Bat Echolocation Research

tools, techniques and analysis

EDITORS

R. Mark Brigham, Elisabeth K. V. Kalko, Gareth Jones,
Stuart Parsons, Herman J. G. A. Limpens

Austin, Texas
2002

BAT CONSERVATION INTERNATIONAL
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National Fish and Wildlife Foundation

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SYMPOSIUM SPONSORS

Hosted by Bat Conservation International
Funded by National Fish & Wildlife Foundation

CITATION

Brigham, R. M., et al., eds. 2004. Bat Echolocation
Research: tools, techniques and analysis. Bat
Conservation International. Austin, Texas

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TABLE OF CONTENTS

BAT DETECTOR LIMITATIONS AND CAPABILITIES

Applications for bat research

ACOUSTIC INVENTORIES

Ultrasound Detection: Basic Concepts

ULTRASOUND SPECIES IDENTIFICATION

Field and Laboratory Applications

RESOURCES, RESEARCH, AND STUDY USING BAT DETECTORS

Future Directions

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Donald Redfield Griffin

1915 - 2003

DONALD REDFIELD GRIFFIN coined the term "echolocation" to describe how bats use echoes of sounds they produce to locate objects in their path. This eye-opening discovery about animal behavior spawned a new field of research that attracted colleagues from animal behavior, neurobiology, signal theory, anatomy, and physiology.

Don died on November 7, 2003. He is survived by his work. He left the world of biology a much richer place. To appreciate Don's contribution, read his 1958 classic, *Listening in the Dark*. Do so after you think you have discovered something new about bats and echolocation. Usually, you will find that Don had visited your new discovery somewhat earlier.

I first met Don at the bat meetings in New Orleans in 1973, then spent several months with him while on sabbatical leave in 1976. In 1980, we worked together in the field in Australia. I especially remember his review of a paper I had submitted about the evolution of echolocation. True to form, he was critical, engaging, challenging, and supportive.

In November 2002, he presented a paper about feeding buzzes at the bat meetings in Burlington, Vermont. Once again, Don took us to the magic well of echolocation. It is his well, but he always shared it – and he will ever be with those who go there.

M. BROCK FENTON

INTRODUCTION

IT IS MY PLEASURE TO INTRODUCE YOU TO THIS VOLUME, the outcome of the Bat Echolocation Symposium and Tutorial held April 15-17, 2002, in Austin, Texas. The symposium grew largely from the vision of Lars Pettersson and Herman Limpens, but it could not have occurred without direct financial support of the National Fish and Wildlife Foundation and the organizational efforts of Bat Conservation International, which hosted it.

The symposium brought together leading authorities on bat echolocation and the detector systems used to study these fascinating animals. The focus was to evaluate the applications, capabilities, and limitations of equipment designed to detect and analyze bat-echolocation calls. This volume provides a formal record of the symposium. I hope it will be of considerable use to participants and other investigators already familiar with the use of bat detectors. More importantly, however, I hope these reports will stimulate and guide the uninitiated to consider the ways and means of learning about the world of bats through their echolocation calls. Bats harbor many secrets they have yet to give up. This was strongly reaffirmed in my mind as I listened to the presentations and read the papers contained in this volume. Hopefully, this collection of papers will help improve the techniques used to study bats through their echolocation calls so more of their secrets will be revealed.

The volume contains 23 papers submitted by authors who gave oral presentations at the symposium. All manuscripts were reviewed for merit, clarity, and accuracy by at least one reviewer, with the understanding that rejection was not possible. I sincerely thank Gareth Jones, Elisabeth Kalko, Herman Limpens, and Stuart Parsons for rapidly and thoughtfully reviewing the manuscripts. Their comments and suggestions substantively improved what you will read on the following pages. I then edited each manuscript for clarity and brevity.

I believe the resulting papers provide a good overview of the diversity of work being done on bat echolocation and the viewpoints of those involved in that effort. Some of the papers are, strictly speaking, less "scientific" and more "opinion" than others. This should not detract from their usefulness or from the goal of motivating people to consider other ways and means of doing more and better work. The volume concludes with brief overviews by Bill Rainey and Gareth Jones, who nicely summarize where we got to over the three days.

Donald Griffin, the codiscoverer of bat echolocation – and the person who coined the term – attended the symposium and contributed a paper to this volume. Fully 65 years after he coauthored the initial report describing bat echolocation by these animals, Don was still working to unlock the lingering secrets. This speaks to the dedication of the man and the tenacity with which the animals are holding out on us. We were deeply saddened to learn of his passing in November 2003 and feel it fitting that this volume should be dedicated to him.

I candidly confess that while I was thrilled to be invited to the symposium, I was never sure that my limited expertise merited the honor. For that reason, I was more than willing to serve as editor for this volume. My foremost goal was to make all the manuscripts as clear as possible so they would be as useful as possible. Only the readers can judge if that goal was met.

My second goal was to have the editorial process proceed quickly so that the final product was produced in as timely a fashion as possible. I thank the reviewers and the authors for putting up with my constant flogging and Bob Locke at BCI for undertaking the copyediting and production process. Brian Keeley was the principal logistical coordinator of the symposium. He deserves hearty thanks for seeing to it that a most wonderful mob was brought together. Julie Jenkins and Janet Tyburec of BCI also played significant roles in helping to organize and facilitate a great meeting. I am most grateful to Allyson Walsh, who got the publication process off the ground. She helped me immeasurably and I owe her a sincere debt of gratitude.

But most of all, thank you, Don, for getting us into the game.

R. MARK BRIGHAM

Section 1

BAT DETECTOR LIMITATIONS AND CAPABILITIES

APPLICATIONS FOR BAT RESEARCH

BAT NATURAL HISTORY AND ECHOLOCATION

M. BROCK FENTON

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Today our view of the natural history of bats is inextricably linked to echolocation even though not all bats echolocate and not all bats use echolocation in the same way. The availability of commercially produced, inexpensive bat detectors is one reason for the explosion in data linking echolocation with the biology of bats. Among microchiropteran bats there are two dichotomies in echolocation behavior: 1) species using high intensity versus low intensity calls; 2) species producing calls at low versus high duty cycle. Furthermore, microchiropterans use a wide range of frequencies in echolocation, from around 10 kHz to over 200 kHz. Echolocation by bats is not synonymous with ultrasound. Limited range and information leakage are two important disadvantages of echolocation. It is becoming increasingly obvious that echolocation calls can simultaneously serve a communication role in bats. The implications for bats of these variations in echolocation behavior are reviewed.

Key words: bat detectors, behavior, communication, duty cycle, information leakage

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INTRODUCTION

Today our view of the natural history of bats is inextricably linked to echolocation. It was not always so and, for all of the magical appeal of echolocation (Griffin 1995), there was a time lag of about 30 years between the publication of the first papers about echolocation in 1939 and any groundswell of research activity there (Figure 1 in Grinnell 1980). Paralleling this lag was the time it took for textbook and review literature to document the diversity of echolocation behavior in bats. By the end of the 1970s, field studies of bats and their sounds became more prevalent in the literature and it took still more time for biologists to realize that there was more to bats than echolocation (and more to echolocation than bats).

Instrumentation was a bottleneck in the development of research on echolocation. Initially the lack of equipment for working with sounds beyond the range of human hearing (ultrasonic) hindered the development of this discipline. Only when such systems were readily available commercially and at prices within the reach of the "average" biologist, did research on the natural history of bat echolocation begin in earnest. Today we are faced with an embarrassment of riches when it comes to bat detectors, and it can be hard to remember that this is a relatively new development. In 1980, when Gary Bell and I submitted a manuscript describing species-specific echolocation calls, one reviewer remarked that the information was interesting but of limited value because the necessary equipment was not readily available to others.

Today we know that not all bats echolocate. We also know that all echolocating bats do not use echolocation in the same way, or even use the same approach to echolocation. An undetermined number of species of *Rousettus* use tongue clicks as echolocation signals, while all other bats appear to use vocalizations produced by the vocal cords (Popper and Fay 1995). Some bats use high intensity (>110 dB sound pressure level [SPL] @10 cm) echolocation calls, mainly species hunting flying prey or prey moving on the water's surface (e.g., Kalko 1995; Kalko et al. 1998). Other bats produce low intensity (60-80 dB SPL @ 10 cm) echolocation calls that appear to serve in general orientation while sounds from prey are used to detect, locate, and assess targets. But some bats using low intensity echolocation calls also produce feeding buzzes (e.g., *Myotis bechsteinii* and *Myotis nattereri* – H. Limpens, pers. comm.; *Megaderma lyra* – Ratcliffe, pers. comm.). Some flowers have ultrasonic nectar guides to steer bats towards nectar and pollen (von Helversen and von Helversen 1999; von Helversen et al. 2003). Will we find the same structures in fruits? It is possible that the erect, spike-like fruits of *Piper* species or the U-shaped fruits of other plants could aid bats in localizing them (Thies et al. 1998). Echolocation is most obviously connected to the foraging behavior of bats, but it is also involved in communication and influences access to roosts.

Most species of echolocating bats separate pulse and echo in time and cannot tolerate overlap between the outgoing pulse and returning echo (Fenton et al. 1995; Kalko and Schnitzler 1993; Schnitzler and Kalko 2001). Typically, calls are relatively short, much shorter than the intervals between calls. Since these bats produce signals about 10% of the time, they are "low duty cycle." Other bats (rhinolophids, hipposiderids, and the mormoopid *Pteronotus parnellii*) separate pulse and echo in frequency (Fenton et al. 1995; Schnitzler and Kalko 2001). These species can tolerate overlap between pulse and echo, and they produce signals 40-80% of the time (at high duty cycle). Their calls are long relative to the intervals between calls.

The echolocation calls of most species of bats are "ultrasonic," by definition, beyond the upper limit of human hearing (arbitrarily 20 kHz). Bats use a range of frequencies in their echolocation sounds, from very high-pitched (*Cloeotis percivali* – calls dominated by sounds >200 kHz), to relatively low-pitched (*Euderma maculatum* – calls dominated by sounds ~ 10 kHz), which are not ultrasonic because they are readily audible to most people. But *E. maculatum* is not an exception; many other species use echolocation calls that are readily audible to humans. A sampling from the list includes *Tadarida teniotis* (Russo and Jones 2002), *Otomops martiensseni* (Fenton et al. 2002), *Nyctinomus australis* (Churchill 1998), *Mops midas* (Fenton et al. 1998), *Eumops perotis*, and *Nyctinimops macrotis* (Harvey et al. 1999). Bats producing echolocation calls that are audible to people have now been reported from many locations, but too often biologists speak of bats using "ultrasonic echolocation." Ultrasonic is not synonymous with echolocation, whether we observe the behavior in bats, birds, or toothed whales.

Shorter wavelengths mean that high-frequency echolocation calls have the potential to give an echolocating bat more detail about its target than lower-frequency calls (Griffin 1958; Simmons and Stein 1980). High-frequency calls have the disadvantage of being more subject to atmospheric attenuation than lower-frequency calls (Griffin 1971; Lawrence and Simmons 1982). Atmospheric attenuation and spreading loss combine to limit the movement of sound waves in air, reducing the effective range of echolocation. The frequencies dominating the echolocation calls of most aerial-feeding bats (20 to 60 kHz) probably represent a compromise between attenuation (range) and detail about targets (Fenton et al. 1998).

TWO DISADVANTAGES OF ECHOLOCATION

Range is the first significant disadvantage of echolocation. Kick (1982) demonstrated that using echolocation, an *Eptesicus fuscus* first detects a 19 mm diameter sphere at a distance of 5 m. This study provided the first real data about the short range of echolocation. Short effective range was predictable from what is known about the physics of the movement of sounds in air, and supported by field observations (e.g., Kalko and Schnitzler 1989, Kalko 1995). Observations of the reaction times (distances) of echolocating bats in the field suggest operational ranges of 2-10 m. At a flight speed of 5 ms, ranges of 2 to 10 m correspond to 400 ms and 2000 ms between detection of and contact with a stationary target. Echolocation in air is a short-range operation, and comparable data from dolphins and porpoises (Au 1993), indicate that the same applies to echolocation in water.

Information leakage is the second big disadvantage. An echolocating *Myotis lucifugus* searching for prey produces 110 dB (SPL @ 10 cm) 4-ms-long signals at 50-ms intervals, continuously broadcasting its position and course to listeners that can hear signals dominated by sound in the 40 to 80 kHz range. This information may be used by conspecifics (see below), or by potential prey as many insects have bat-detecting ears (Miller and Surlykke 2001). Many situations in electronic warfare repeatedly demonstrate the high potential cost of using active systems such as radar or sonar to assess one's surroundings. The same costs, namely revealing presence, course, and identity, apply to echolocating bats.

BATS' USE OF ECHOLOCATION

We know most about echolocation in aerial-feeding bats. *Eptesicus fuscus* and probably any species in the genera *Eptesicus*, *Nyctalus*, *Lasiurus*, *Chalinolobus*, or *Pipistrellus*, as well as many other vespertilionids could be considered as "typical" aerial feeders, along with emballonurids or molossids (reviewed by Schnitzler and Kalko 2001). Aerial feeders use echolocation to detect, track, and assess airborne targets, typically flying insects. These are low duty cycle species, but high duty cycle species may also use echolocation to find flying prey (Fenton et al. 1995).

The same basics of signal design and pulse repetition rate apply to some bats, which take prey from surfaces. The best examples are *Noctilio* and *Myotis* species that use echolocation to detect the ripples associated with prey (usually fish) swimming close to the surface of the water (e.g., Boonman et al. 1998; Kalko and Schnitzler 1989; Schnitzler et al. 1994; Siemers et al. 2001). The same applies to *Megaderma lyra* hunting frogs in water (Marimuthu et al. 1995).

Many gleaning bats, animal-eating species taking prey from surfaces, use cues such as the sounds of movement or advertisement to detect, locate, and assess targets. Gleaners typically use low intensity, short (< 2 ms long) broadband echolocation calls. A few gleaners, *Macrotus californicus* (Bell 1985) or *Cardioderma cor* (Ryan and Tuttle 1987) for example, appear to use vision when there is sufficient light. *Desmodus rotundus* also appears to be able to use vision in some situations (Manske and Schmidt 1976). Some other species, notably *Myotis emarginatus*, switch between gleaning and aerial feeding, adjusting their echolocation behavior accordingly (from low intensity while gleaning to higher intensity for aerial feeding (Krull et al. 1991; Schumm et al. 1991). Even in this situation, however, *M. emarginatus* does not produce echolocation calls as intense as those of other species (e.g., *Pipistrellus pygmaeus* – Limpens, pers. comm.).

The role that echolocation plays in the natural history of plant-visiting bats, whether they eat fruit and leaves or nectar and pollen, has not been clear. In 1999, von Helversen and von Helversen showed how some flowers have ultrasonic nectar guides to assist New World nectar-feeding bats in locating nectar. Earlier, two other studies suggested a role for echolocation in finding fruit by *Phyllostomus hastatus* (Kalko and Condon 1998) and *Carollia* species (Thies et al. 1998). Before these works were published, the short, low intensity, multi-harmonic echolocation calls of plant-visiting bats did not offer much promise in the detection or evaluation of food. The same absence of the lack of a clear role for echolocation applies to the blood-feeding vampire bats.

The signals that an individual uses for echolocation (to collect information about its surroundings) are also available to others. Playback experiments have demonstrated how echolocation calls can serve a communication function in a variety of species such as *Myotis lucifugus* (Barclay 1982), *Euderma maculatum* (Leonard and Fenton 1984), and *Lasiurus borealis* (Balcombe and Fenton 1988). There is evidence that in *Otomops martiensseni*, calls originally associated with echolocation may actually serve more for communication (MBF unpublished observations).

A bat's communication repertoire typically includes other signals. Acoustic signals dedicated to communication are longer in duration than echolocation calls (and therefore of minimal value in echolocation because of pulse-echo overlap). Some are individual-specific and serve in food location and group maintenance (Boughman and Wilkinson 1998), while others are interspersed with echolocation calls and associated with mating (Barlow and Jones 1997). Bats appear to simultaneously use some vocalizations for echolocation and others for social purposes, placing interesting demands on their auditory systems because it necessitates simultaneous processing of different kinds of auditory information. In many species, communication also involves the use of olfactory signals (Bouchard 2001). People whose first language is English should make a special effort to keep abreast of data published in other languages, particularly in the area of echolocation and social behavior (e.g., Limpens et al. 2001).

THINGS FOR WHICH ECHOLOCATION PROBABLY DOES NOT WORK

The short effective range of echolocation appears to preclude using this mode of orientation in navigation and other long-distance (> 20 m) perceptions. For example, a *Macrotus californicus* would first see a tree-sized object at over 1 km and a June beetle-sized insect at18 m, while an *Eptesicus fuscus* would only see the June beetle at 1 m and the tree at about 300 m (Bell and Fenton 1986). *Phyllostomus hastatus* deprived of vision appear to use some combination of spatial memory and echolocation to orient themselves and find "home" (Williams et al. 1966). *Eptesicus fuscus* use the glow of sunset in general orientation (Buchler and Childs 1982). We do not know what cues bats that make long distance migrations use, but certainly vision offers more promise in these situations than echolocation because of effective range. Verboom et al. (1999) have demonstrated how *Myotis dasycneme* used echolocation to collect data about their general surroundings.

Low-intensity calls dominated by higher frequencies should translate into a very short effective range, probably substantially less than the 5-m range for 19-mm diameter targets demonstrated for *Eptesicus fuscus* (Kick 1982). Mind you, I would also have said this about flower-visiting bats prior to the publication of reports by von Helversen and von Helversen (1999) and von Helversen et al. (2003) or fruit-eating bats before reports from Thies et al. (1998) and Kalko and Condon (1998).

ECHOLOCATION AND OUR VIEW OF BATS

Curiosity about echolocation and the appearance of affordable equipment for studying it have meant that research on bat sounds has dominated our perception of these animals. From the outset, however, Griffin (1958) and others (e.g., Suthers 1970) repeatedly reminded us that bats can see and, like other mammals must use a range of channels (visual, olfactory, acoustic, tactile) in communication (Altringham and Fenton 2003). Perhaps the most interesting frontier for bat biologists is the means by which bats integrate the information they collect through the various senses.

Echolocation has changed our view about the diversity of bats. Differences in echolocation calls backed up by molecular data led Barrett et al. (1997) to ascertain that bats called *Pipistrellus pipistrellus* really were two species. We can expect other such examples to emerge as we increase our databases documenting variation in echolocation calls (Obrist 1995).

RECOMMENDED READING

To appreciate the entanglement of the way we see bats and echolocation, I strongly recommend that you read two classic books, Glover M. Allen's *BATS* (1939) and Donald R. Griffin's *Listening in the Dark* (1958). The first book will take you back to our view of bats, their biology and natural history, in the days before echolocation. The second will change your view of bats by making echolocation front and center. Then read André Brosset's classic *La Biologie des Chiroptères* (1966) to see how echolocation begins to intertwine with our view of the biology and natural history of bats.

ACKNOWLEDGEMENTS

I thank the organizers of the symposium for including me on the program as well as those who read and commented on earlier versions of this manuscript, including Rafa Avila-Flores, Enrico Bernard, Stefania Biscardi, Herman Limpens, Brian Keeley, Liz Reddy and Hannah ter Hofstede. My research on bats has been supported by research grants from the Natural Sciences and Engineering Research Council of Canada.

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THE PAST AND FUTURE HISTORY OF BAT DETECTORS

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What we can learn about the echolocation abilities of bats has been limited by the sensitivity of microphones; and some of their high-frequency sounds cannot be detected under many conditions, even with the best instruments currently available. For example, sensitive heterodyne bat detectors sometimes fail to detect feeding buzzes from *Myotis lucifugus* on early summer evenings when they are obviously catching insects. By mounting the bat detector on a long pole and using infrared video showing the bat and the insect it captured, we found that buzzes were detectable only if the microphone was within one or two meters of the bat. Yet at other times buzzes could be detected from the same bats in the same location at distances of ten meters. Many neotropical bats emit much fainter sounds than *M. lucifugus*, and there is some evidence that shrews and laboratory rats use echolocation, although it has been difficult to detect whatever sounds they may be emitting. There is an "apparatus threshold" which is ordinarily well above the auditory thresholds of bats and shrews, so that there is a sizeable intensity range where we cannot yet determine whether sounds are being used for echolocation or communication.

Key words: bat detectors, echolocation, equiptment, insect capture by bats, microphone sensitivity, *Myotis lucifugus*

PAST HISTORY

To study the echolocation abilities of bats it is first necessary to detect their orientation sounds. The fact that bats make ultrasonic sounds was discovered by means of G. W. Pierce's "sonic amplifier," which was a heterodyne detector with an audio output constructed by modifying an AM radio receiver (Pierce and Griffin 1938). This apparatus had been developed to study the sounds of insects; its circuit is described by Noyes and Pierce (1937) and the microphones by Pierce (1948). The essential first element in any apparatus for detecting bat sounds is the microphone. Pierce used magnetostriction transducers and Rochelle salt crystals as microphones; the latter were somewhat more sensitive, but neither was calibrated nor nearly as sensitive as microphones developed later. Pierce's apparatus displayed the heterodyne output graphically, but its temporal resolution was not adequate to measure the duration of the ultrasonic orientation sounds used by bats of the family Vespertilionidae. It could record pulse repetition rates and revealed that the interpulse interval dropped by about half when *Myotis lucifugus* avoided small wires (Galambos and Griffin 1942). Furthermore it enabled us to demonstrate that emission and reception of orientation sounds were necessary for obstacle avoidance (Griffin and Galambos 1941; Griffin 1958).

The next step was to use the Western Electric 640 steel diaphragm condenser microphone, which was almost identical to the Bruel and Kjaer one-inch microphone, and to display waveforms on a cathode ray oscilloscope. This apparatus was AC-operated, bulky, and considered far too delicate for use in the field. It showed, however, that the bats' orientation sounds were FM chirps sweeping through about one octave (Griffin 1946). The sensitivity of these microphones is nearly constant up to about 12 kHz but then drops by about 12 dB per octave. Yet the internal noise level is low enough that components of bat sounds up to at least 120 kHz can be displayed on the oscilloscope. Filter circuits can correct for the declining sensitivity, but signal-to-noise levels necessarily drop as the microphone becomes less sensitive at higher frequencies.

When I first studied the orientation sounds of *Eptesicus fuscus* in the field in 1951, we needed a station wagon to carry the necessary apparatus, which included a gasoline engine-driven AC generator, oscilloscope, modified movie camera (to record the oscilloscope displays), and a long, deep parabolic horn to increase microphone sensitivity. The displays were entirely different from anything seen in the laboratory, a mine tunnel, or nursery colonies in buildings. Since it was impossible to watch at the same time both the flying bat and the oscilloscope in the back of a station wagon, we needed an audible indicator of the bat sounds. The rough and ready solution was to connect the amplified signal from the microphone to the second detector stage of a portable AM radio. This was a very clumsy bat detector, but it worked so well that I used it for field work for several years because the other necessary apparatus was so bulky that it was not worth the trouble to build a smaller version. This system allowed me to discover that when pursuing insects (or tossed pebbles), *E. fuscus* increased its pulse repetition rate by ten or twenty fold. This was a most striking change from the rough doubling of repetition rate that Galambos and I (Galambos and Griffin 1942) had found when bats were dodging small obstacles.

As is now well known, the typical pattern is a roughly constant interpulse interval during the search phase, a gradual decline in this interval during the approach phase, ending with a burst of very short duration sounds with interpulse intervals of only a few milliseconds. With most bat detectors, this sounds like a buzz and is called a feeding, or terminal, buzz (Griffin 1958).

The next major advance was to take essentially the same apparatus to Panama in 1953 to make the first investigation of echolocation by Neotropical bats. A car or truck was still required to carry the apparatus to field sites, and an outboard motor-powered dugout canoe was needed to reach the best location on the upper Chagras River, where we observed *Noctilio leporinus* catching fish. To reduce noise from the generator, it was left on shore behind trees, with the power cord forming a long umbilicus from a tree out to the canoe loaded with all the electronics and a high-speed camera to record the oscilloscope displays. The same crudely adapted AM radio bat detector worked well for many Neotropical bats, but it was puzzling that the very abundant small fruit-eating bats such as *Carollia perspicillata* produced faint clicks from the radio but no clear signals on the oscilloscope. However, these "whispering bats" collided with obstacles when their ears were plugged, showing that they were echolocating. Their orientation sounds were too faint to rise clearly above the background noise level of our apparatus.

We now know that different bats use widely varying types of orientation sounds, varying greatly in frequency and intensity. Many of our ideas about bat echolocation have been determined by the species with which we are familiar. If in the 1930s, Pierce, Galambos, and I had studied only *Carollia*, we would never have discovered that bats emit ultrasonic orientation sounds. And if Spallanzani and Jurine had observed some of the Molossidae that emit orientation sounds in the frequency range audible to human ears, they would have discovered bat echolocation in the 1790s.

The landmark paper by Kühl et al. (1954) showed how to build plastic diaphragm microphones with roughly constant sensitivity to 100 kHz and beyond. Novick used these microphones to extend and improve the 1953 studies of Neotropical bats; and the plastic diaphragm microphones sufficed to detect orientation sounds from all bats he studied. But for some species, the emitted intensities were so low that orientation sounds could be detected only when the microphone was close to the bat (Griffin and Novick 1955). This experience taught us that what we can learn about sounds of bats and other animals is often limited by the internal noise level of microphones, which limits their sensitivity to low-level sounds. The Knowles microphones used in many modern bat detectors are basically similar.

In the late 1950s, the MIT Lincoln Laboratory developed a true bat detector that was portable (only about 25 by 10 by 20 cm in size and weighing approximately a kilogram). It employed a plastic diaphragm microphone and was an envelope detector, producing as an audio signal the envelope of the ultrasonic bat pulse (McCue 1961). Although long since superseded by modern bat detectors, this instrument was a great improvement over anything I had used previously. Somewhat later, leak detectors and simple bat detectors based on crystal microphones tuned to about 40 kHz proved useful for detecting those bats whose orientation sounds sweep through 40 kHz. Recently, Tony Messina and others have been developing relatively inexpensive bat detectors based on this type of microphone, which is now commercially available for only a few dollars. These detectors certainly make it possible to enter a bat's acoustic world, but they cannot detect the full range of frequencies used by bats for echolocation.

Once ultrasonic waveforms have been converted by a microphone to electrical signals, these can be converted to audible sounds by several methods that will be described by Lars Pettersson (this volume) and other participants in this symposium. Since the orientation sounds of all bats have durations of only a fraction of a second, the output of most bat detectors sounds like a click. This suffices for detecting bats and for analyzing pulse repetition rate. But the full waveform, including harmonics, is probably used by the bats under some conditions and is therefore important for understanding their behavior. Photography of cathode ray oscilloscope displays provided only limited indication of harmonic content and was superseded when devices capable of tape-recording ultrasonic frequencies became available. These in turn are being replaced by digital recording. It is now possible, and highly desirable, to record and analyze the full frequency spectrum of the sounds bats use for echolocation and communication.

Commercial development of increasingly small and versatile bat detectors has led recently to many types only a few of which I am familiar with. Other authors will bring the art and practice of bat detecting up to date. I would therefore like to turn to some general considerations about the potential future of apparatus capable of helping us learn more about echolocation and communication of bats under natural conditions.

DESIRABLE ADVANCES

Students of biology and behavior strive to learn all they can about the sounds that animals use for both echolocation and communication. As splendid as currently available instruments are, there are still many situations in which what we can learn is limited by the sensitivity of the microphones. For example, the fact that the orientation sounds of many neotropical bats are only barely detectable by bringing the microphone quite close to the animals obviously limits what we can learn about their natural behavior. There is suggestive but ambiguous evidence that shrews and possibly other terrestrial mammals may use a limited form of echolocation (Buchler 1976). If they emit any orientation sounds, these are difficult to detect – perhaps because they are too faint to rise above the internal noise level of available microphones. At present it is not possible to detect broadband sounds close to the auditory thresholds of bats and other mammals. There is thus a sort of "blind area" in frequency and intensity space where animals may emit and hear sounds that we cannot yet reliably detect.

One recent example of these limitations arose when listening to *Myotis lucifugus* with QMC Mini and Pettersson D-100 heterodyne bat detectors in early summer. I was surprised that on some evenings, but not others, I heard very few buzzes, although I was using the same bat detector in the same places under what appeared to be identical conditions. These bats certainly must have been hungry in the early evening, and many were pregnant or nursing females. Could they be catching insects without the familiar feeding buzzes, or was I failing to detect buzzes that were being emitted?

Once this question was raised, clearly some independent evidence of insect pursuit other than the feeding buzz was needed. Gregory Auger and I have therefore worked out procedures for obtaining video by near-infrared illumination showing insect captures by *Myotis lucifugus* with heterodyne bat detector output on the same sound track. In some cases, an insect was clearly captured but no feeding buzz was detected. Furthermore, there was often little or no approach phase in which the interpulse interval shortened progressively. We could not be sure whether the bat was not emitting approach-phase and buzz pulses or we were not detecting them. The simplest way to improve the signal-to-noise ratio was to mount a Pettersson D-100 bat detector on a long pole, which we could sometimes hold fairly close to where the video showed that a bat caught an insect. In the summer of 2002, we used two video camcorders, one aimed vertically and the other horizontally. The second camcorder was not sensitive enough to show the insects, but it did show whether the capture occurred at approximately the same distance from the first camcorder as the bat detector. In all cases when the bat was within about 2 meters of the microphone and was heading roughly toward it, we did record a buzz. However, the intensity of approach phase and buzz was sometimes much lower than in other observations of the same bats with the same equipment.

To explore the lower range of emitted intensity of bat sounds we need microphones that can reliably detect sounds of lower intensities. This special problem of seemingly buzzless catches by *M. lucifugus* may not be the only situation where we have been limited by microphone sensitivity. How might microphone sensitivity be improved? As plastic diaphragm microphones are made larger in area they are more sensitive, but also more directional, that is, sensitive only over an increasingly narrow beam perpendicular to the plane of the diaphragm. Could the plastic and the backplate be dome-shaped to avoid too narrow a beam of sensitivity? Are there other inherently superior types of transducers than the plastic diaphragm condenser microphones of the basic design first developed by Kühl et al. (1954)? Laser vibrometry detects very small motions. It is possible that this could this be adapted to detect fainter movements resulting from impinging sound waves. At first thought, such an apparatus would seem delicate, expensive, and likely to be limited to laboratory use. But that was true for many years of the best available microphones, which made it possible to learn the basic aspects of bat echolocation.

Finally, it is beginning to be feasible to use differences in arrival time of a bat's sounds at multiple microphones to determine its position in space (Holderied 2002). This opens up exciting new possibilities of analyzing flight paths and behavioral interactions between bats. It also may make it possible to study the directional pattern of sound emission by flying bats during natural behavior, including insect hunting. Emitted beam patterns seem to vary somewhat during the octave of downward frequency change in each of the brief orientation sounds, but it has been extremely difficult to measure this with flying bats. It is possible that bats might vary frequency-dependent beam patterns to adapt them to their current behavior. For example, a broad beam must be best for detection of insects, but once one is located might the bat shift to a narrower beam aimed at the target of interest? It is almost impossible to test such a hypothesis without some way to measure the bat's position in three-dimensional space and the direction in which its head is pointed. As with many other aspects of echolocation behavior, I suspect that there are significant surprises waiting for enterprising investigators to discover.

ACKNOWLEDGEMENTS

I wish to thank The Rockefeller and Harvard Universities for financial and administrative support, Peter Auger and his students for assistance, and especially Gregory Auger for technical collaboration.

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THE PROPERTIES OF SOUND AND BAT DETECTORS

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When an ultrasonic call is transmitted through the air, captured by the microphone of a bat detector and transformed into audible sound, there are several factors that influence the characteristics of the resulting sound. As sound waves travel through the air, reflections, atmospheric absorption and geometric spreading are some of the factors that alter it. The microphone of the bat detector can also affect the sound, e.g., by exhibiting a more or less non-ideal frequency response and directional sensitivity. The properties of the capacitance, electret, and piezoelectric microphone types are discussed. Bat detectors make use of different ultrasound conversion techniques, resulting in different types of sound. I describe the heterodyne, frequency division and time expansion bat detector principles and the advantages and drawbacks of each type.

Key words: bat detectors, frequency division, heterodyne, sound transmission, time expansion, ultrasound detectors, ultrasound microphones

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INTRODUCTION

Bat detectors convert inaudible, ultrasonic signals into audible signals and thus are important tools for studying bats. There are several techniques that can be used to transform ultrasonic signals into audible signals. Here I describe the heterodyne, frequency division, and time expansion techniques.

In order to register ultrasonic signals, bat detectors require microphones sensitive to such high frequencies. The most common microphone types, the capacitance, electret, and piezoelectric types, are described. As the ultrasonic sound wave propagates through the air, several physical phenomena will affect it. I discuss the most important phenomena.

THE PROPAGATION OF SOUND IN AIR

Sound waves can be described as variations in pressure propagating away from the sound source with a certain velocity. When we want to capture and study the sound at some distance from the sound source, it is important to consider how the sound wave may be changed as it propagates through the transmission medium.

There are a number of physical phenomena that may affect the sound wave (see Bradbury and Vehrencamp 1998, Gerhardt 1998, Hassall and Zaveri 1979). Examples of such phenomena are geometric spreading, reflection, absorption, refraction, scattering, and Doppler shift.

Geometric spreading causes the sound intensity to decrease with the distance from the source. In the far field, the sound intensity is inversely proportional to the square of the distance from the source, i.e., it is decreased by 6 dB per doubling of distance. This is a simple consequence of the fact that from a point source, the sound energy spreads out equally in all directions. Hence, the spreading loss caused by geometric spreading can be easily calculated and is "harmless" in the sense that all frequencies are affected equally.

The simple geometric laws of reflection used in optics are valid also for acoustic waves, provided the reflecting surface is large and the surface roughness is small relative to the wavelength of the sound wave. An incident wave with a certain grazing angle will give rise to a reflected wave leaving the surface with the same angle. The direct and the reflected waves will interfere depending on the frequency, distance, and reflection coefficient. The interference in a certain situation may be constructive or destructive, which may result in the disappearance of certain frequencies.

For ultrasonic signals traveling near the ground, vegetation and general surface roughness have large dimensions relative to the wavelength of the sound, so any reflections will be rather diffuse. Hence, such signals can propagate near the ground surface almost without being affected.

One exception to this is a calm water surface, which may give rise to well-defined reflections. Hence, bat calls recorded as the bat flies over a water surface sometimes exhibit significant amplitude variations and "missing frequencies."

It is worth noting that reflections occur not only at the boundary between two different media, but anywhere that the acoustic impedance changes, e.g., at the interface of air masses with different temperature or humidity.

As sound waves propagate in a medium, a certain amount of energy is lost to the medium. Collisions between the molecules in the medium, resulting in dissipation of sound energy into heat, and the absorption of energy within the molecules of the medium are two important factors that contribute to "medium absorption." The medium absorption in air varies with the temperature and the humidity of the air, as well as the frequency of the sound. Temperature and humidity normally change relatively slowly, while the signal frequency may change rapidly. The frequency dependence of absorption is thus of particular importance here.

At 10 kHz, the attenuation due to absorption in air is about 0.1 dB per meter, increasing to approximately 10 dB per meter at 200 kHz. Since the actual attenuation also depends on temperature and humidity, it is difficult to apply precise corrections to account for this effect. The attenuation of higher ultrasonic frequencies makes it difficult for bats to use high-frequency calls for echolocation over long distances.

If the sound source is moving relative to the receiver, Doppler shift will change the received frequency. As an example, if a bat flies towards the observer with a velocity of 5 ms^{-1}, emitting a call at a frequency of 50 kHz, the received frequency will be about 50.7 kHz.

Other factors that affect sound propagation include refraction (deflection of a sound wave caused by a change in the propagation velocity of the medium) and scattering (redirection of the sound wave as its passes an object).

ULTRASOUND MICROPHONES

There are many different types of microphones. However, for ultrasonic applications the most common are the capacitance, electret, and piezoelectric types.

A capacitance microphone consists of a metal backplate and a thin metalized, plastic membrane. The membrane (or diaphragm) is stretched over the backplate to make the two parts form the two plates of a capacitor. As the sound waves make the membrane move, the capacitance changes, and these changes are converted into a proportional voltage.

A solid-dielectric capacitance microphone uses a slightly uneven (e.g., grooved) backplate, with the membrane stretched over the backplate and in direct contact with it. The grooves are required in order to allow the membrane to move with the sound waves. In an air-dielectric capacitance microphone, the membrane is mounted at a certain short distance over the backplate.

The most common type of capacitance microphone in bat detectors is the solid-dielectric type, due to its high sensitivity and relative robustness. The frequency response of a capacitance microphone is usually quite flat, i.e., it is equally sensitive at different frequencies. For a well-designed microphone, the frequency response is typically flat within ±4 dB up to 150 kHz or higher.

A capacitance microphone requires a relatively high polarizing voltage, usually 100-200 volts. The polarizing voltage is applied over the microphone element, which, in the case of a solid-dielectric microphone, means a very thin barrier. At high polarizing voltages this means an increased risk of discharges, which of course generates high-level noise from the microphone. Particularly in high humidity, the noise level of this microphone type may increase.

An electret microphone is similar in design to the capacitance type. The former does not require a polarizing voltage though, since the dielectric material can retain a permanent charge. Most electret microphones are intended for audio use, but they may still have a useable response in the ultrasonic-frequency range. There are also a few types specifically designed to yield better high-frequency responses.

Both the capacitance and electret microphone types have built-in preamplifiers. This is required to convert the high impedance of the microphone element into a lower impedance. The sensitivity of the microphone element and the noise level of the preamplifier are the most important factors that determine the signal-to-noise ratio of the microphone and thus its capability of recording weak signals.

The piezoelectric microphone type is easy to use, rugged, cheap, and sensitive. However, it is a narrowband type of transducer, so it is sensitive only over a very limited frequency range. Standard types have a 3 dB bandwidth of only a few kHz. There are broadband types with a 3 dB bandwidth of about 10 kHz, but this is achieved at the expense of lower sensitivity. The most common piezoelectric microphones use a 40 kHz center frequency, but there are other types available, e.g., 25 kHz and 32.7 kHz. Combining two or more of these results in a wider useable frequency range. This is an alternative for less demanding instruments.

ULTRASOUND CONVERSION TECHNIQUES IN BAT DETECTORS

Bat detectors convert the ultrasonic calls of bats into audible signals, making it possible for humans to hear the sounds. An experienced observer is then often capable of determining what the bat is doing and which species it is. There are a number of different methods to convert ultrasonic signals into audible sounds, the most common of which are heterodyning, frequency division, and time expansion. Each method has advantages and disadvantages, so some bat detectors contain two (or even three) independent conversion systems.

HETERODYNING

Heterodyning is perhaps the most common conversion method. It is a narrowband technique, which means that only a narrow range of frequencies is made audible at a time. The width of the frequency range that is transformed is determined by a filter in the bat detector and is typically about 10 kHz. The tuning control is used to set the center frequency of the frequency range to transform, so setting this at 40 kHz will make frequencies between 35 and 45 kHz audible.

In a heterodyne detector, there is an oscillator, the frequency of which is adjusted with the tuning control. A mixer circuit is used to multiply the oscillator frequency, f_o, with the ultrasonic signal frequency, f_s. This results in a signal consisting of two frequency components, f_s-f_o and f_s+f_o. The latter component is eliminated by passing the signal through a lowpass filter. Provided that f_s is close enough to f_o, the difference f_s-f_o will be audible. By way of example, if the tuning control is set to a frequency of 40 kHz and the frequency of the input signal is 42 kHz, the resulting frequency is 2 kHz, which is clearly audible.

The heterodyne technique results in sensitive bat detectors, i.e., it is possible to detect very weak signals. This is a simple consequence of the narrowband behavior. Since only a small part of the entire ultrasonic frequency range is made audible, only a correspondingly small fraction of the entire full-range noise will be made audible. Hence, the noise level will be low and the sensitivity high.

Bat calls transformed in a heterodyne detector sound different depending on the frequency to which the detector is tuned. At some settings, a "smacking" sound is obtained, while other settings result in a "ticking" sound. These tonal qualities can be used to determine the type of bat call (FM sweep, CF, etc). The approximate frequency of a bat call can also be determined by varying the tuned frequency and listening to the resulting sounds.

The narrow bandwidth of a heterodyne detector means that if the detector is tuned at a certain frequency and a bat call at another frequency is received, the bat call will pass undetected if its frequency is too far away from the tuned frequency to make it audible.

FREQUENCY DIVISION

In contrast to the heterodyning, frequency division is a broadband technique, i.e., the entire ultrasonic range is transformed at all times. The result of this transformation is a frequency being a fixed fraction (e.g., one tenth) of the original frequency. A frequency division detector is less sensitive than a heterodyne detector, so weak calls may not be detected. This is due partly to the fact that all noise from the full frequency range is made audible, resulting in a poorer signal-to-noise ratio, but also because most frequency division detectors have a minimum threshold level, below which they do not produce any output signal. However, the transformed signal of a frequency-division detector contains more information than that of a heterodyne detector and can be used for certain types of sound analysis.

In a frequency-division detector, the original signal is first converted into a square wave, regardless of its original shape. All amplitude information is lost at this stage. The square wave is fed to a digital frequency divider which counts the number of times the high-frequency signal goes from negative to positive (or vice versa) and generates one output cycle for e.g., every ten positive (or negative) zero-crossings. Provided the input signal is a reasonably pure signal, with only one positive zero-crossing per cycle, this results in an output frequency of one-tenth of the input frequency. It should be noted that it is only possible to represent one spectral component at each time with the frequency-division technique. If the original signal is composed of a fundamental frequency and one or more harmonics, only one of them will be transformed. Usually, but not always, the fundamental frequency will be transformed. In cases where a strong harmonic is present, this may be transformed rather than the fundamental. Despite what has been said, the output signal may contain harmonics. However, these are in no way related to the harmonics, if any, of the original signal. They are simply a result of the waveform of the output signal (often a square wave).

Some more advanced frequency-division detectors also process the amplitude information and have this superimposed on the frequency-divided output signal, to give this the same amplitude as the original signal.

Hence, from a frequency-division signal, it is usually possible to obtain information on the fundamental frequency of the original signal. Provided the detector retains the amplitude, the pulse duration and other temporal parameters can also be measured.

A frequency-division detector is not capable of accurately representing rapid frequency changes in the input signal. Frequency variations in the original signal that take place over a period of time less than the time for one cycle of the output signal will not be visible after transformation. For bat calls, this is normally not an issue. However, in an FM-sweep signal it may be difficult to obtain reliable readings of the start and end frequencies.

Signals transformed in a frequency-division detector do not have tonal qualities like those from a heterodyne detector. Nor do they have the extended length of those from a time-expansion detector. Altogether, this means that frequency-division signals are less suitable for instant identification in the field.

TIME EXPANSION

The time-expansion method means that a high frequency signal is recorded with some broadband recording device and then played back at a slower speed. The resulting signal will obviously be longer than the original and the frequency correspondingly lower. The transformation is not made in real time. In a time-expansion bat detector, the signal is digitized and a portion of it is stored in a digital memory, from which it is replayed at e.g. one-tenth of the original speed (e.g., Ahlén and Pettersson 1985, Pettersson 1985 and 1999). Since the signal stored in the memory is the original signal, the transformed signal will contain the same information as this. Hence, time-expanded signals are suitable for any type of sound analysis. The time and frequency axes in the resulting diagrams can easily be adjusted to show the figures of the original signal instead. There are even sound-analysis programs that do this automatically.

The fact that time-expanded signals are stretched out in time makes it possible to hear details in the sound that are not audible with other transformation methods. This is of value also for immediate use in the field.

In order to use a time-expansion detector efficiently, a real-time transformation system is required. That way, the user has control over the bat-call activity in real time as well. For this reason, most time-expansion detectors also have a heterodyne system built-in.

Today there are time-expansion detectors with a storage time of up to a few tens of seconds, which is sufficient for most situations involving bats. For longer recording times, other alternatives are available, such as direct recording with a computer through a high-speed sound card.

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FORAGING HABITS OF NORTH AMERICAN INSECTIVOROUS BATS

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This paper presents an historical overview of efforts to characterize and quantify the foraging habits of selected North American insectivorous bats. Where appropriate, it also highlights methods that have been used to explore the foraging habits of other insectivorous species. What we currently know about the foraging habits of insectivorous bats in North America, and elsewhere, has largely been inferred from dietary analyses, captures of bats using mist nets and harp traps, observations of bats in night roosts, temperature recordings in day and night roosts, recordings of echolocation calls with ultrasonic detectors (bat passes and feeding buzzes), assessment of nightly activity using radiotelemetry, and observations enhanced by light tags, night-viewing devices, and infrared video. Recent advances in the study of foraging habits of insectivorous bats have been enriched by the availability of new technologies and approaches, including advanced ultrasonic detection and analysis, Doppler radar imaging, infrared thermal imaging, and passive integrated transponders (PIT) tags. In addition, ecomorphological analyses and computerized models of wing structure, skull, and dentition have contributed greatly to our understanding of foraging habits of bats. Each method has its own biases, advantages, and disadvantages and may yield different results depending on the species being observed or recorded, the local species richness, density and distribution, body size, age, sex, reproductive condition, type of habitat being monitored, and the sensitivity of the bats to the method(s) being used.

Key words: capture methods, dietary analysis, ecomorphology, observations, PIT tagging, remote sensing, ultrasonic monitoring

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INTRODUCTION

Foraging habits of bats have largely been inferred from the analysis of diet (based on examination of stomach contents and feces and parts of discarded prey), observations of nightly emergence and return times, capture times of commuting, feeding, and drinking bats, temperature recordings in roosts, ultrasonic recordings of commuting and foraging bats, observations of light-tagged bats, and the activity of radio-tagged and PIT-tagged individuals. Information derived by using one or more of these approaches has provided valuable insight into the foraging habitats of North American insectivorous species. Emerging technologies, such as Doppler radar imaging, infrared thermal imaging, 3-dimensional photography and videography, and computer modeling offer exciting new opportunities to characterize the feeding habits of insectivorous bats. When traditional methods are complemented with advanced technologies, new insight into the behavior and ecology of foraging bats is certain to emerge.

Many early studies that attempted to assess the foraging habits of free-ranging insectivorous bats were based on direct observations during periods when ambient light was sufficient to detect flying/feeding, usually during a brief period following sunset. Early naturalists often recorded bats drinking or foraging over water or near the edges of forests, where their silhouettes could be observed against an unobstructed sky. In some cases, bats were shot while they foraged. Fortuitous captures and shooting provided information on diet (stomach contents), and the places and times that some species fed. The availability of Japanese mist nets after World War II made it possible to readily capture bats as they commuted and foraged or sought water to drink (Dalquest 1954; Handley 1967). The development of the double-frame harp trap in the late 1960s (Tuttle 1974), and more recently the 4-frame harp trap (Francis 1989) has opened new opportunities to capture large numbers of bats, especially at caves and buildings (Kunz and Kurta 1988), and in forested regions (Kingston et al. 2000), that would not have been possible using mist nets alone.

Ultrasonic detectors were first developed to evaluate the structure of echolocation calls as bats navigated or fed on insects in the laboratory (Griffin 1958; Pye 1983). Bat detectors became available for field use in the 1960s (Griffin this volume; Pye 1983), but many were not widely available or ideally suitable for field studies. From the mid-1970s until present, field-friendly bat detectors have become increasingly available. In the 1980s several commercial developments made it possible for these devices to be deployed for a variety of purposes, ranging from assessing general activity levels to monitoring activity of certain species in different habitats (Fenton 2000; Fenton 2001). In the 1990s, advances in circuit design, microphones with increased sensitivity to ultrasound, and advanced computer software made it possible to record and store the full range of echolocation-call structures in the field and, in many situations, to identify bats to species (Ahlén this volume; Limpens this volume; Pettersson this volume).

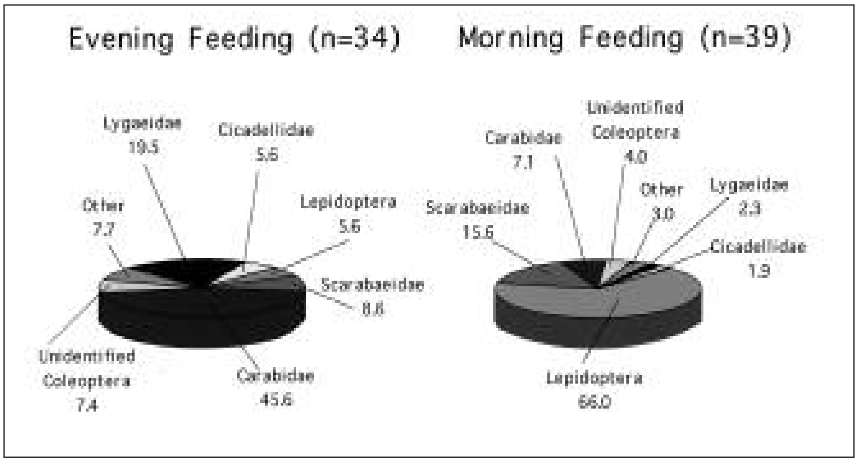

Figure 1: Nightly variation in the diet of the Brazilian free-tailed bat (*Tadarida brasiliensis*) in Texas. The diets of bats from the pre-midnight samples differ significantly from those recorded from the pre-dawn samples (after Whitaker et al. 1996).

Bat detectors offer alternatives to more traditional methods to assess the presence of insectivorous bats, especially in environments and situations where it is not possible to make direct observations or to capture them. Notwithstanding, bat detectors are only one of a number of tools available for investigating the foraging habits of insectivorous bats. As with most devices, biases associated with their limits of detection, uncertainty of discriminating species-specific call signatures, and inability to discriminate individuals by sex, age, and reproductive condition, place limits on the general applicability of bat detection for assessing the full extent of foraging habits of echolocating bats (Hayes 2000). Live captures and other methods (Kunz and Kurta 1988; Kunz et al. 1996) are needed to gain a full understanding of the foraging habits of insectivorous bats.

The purpose of this paper is to review research methods that offer information useful for evaluating and quantifying the foraging habits of insectivorous bats. In this review, I consider how research on the foraging habits of insectivorous bats (e.g., diet, commuting and foraging behavior, night-roosting, energy budgets, etc.) should employ a number of complementary techniques. Use of complementary techniques, including live capture, identification of species in the hand, fecal analysis, radiotelemetry, temperature telemetry, and the like, when used together, can contribute most towards our understanding. However, each capture device or observational method comes with its own limitations and biases.

DIETARY ANALYSES

Most early efforts to characterize feeding habits were secondary to studies designed to document geographic distributions (i.e., dietary data were based largely on specimens destined for museum collections). Additionally, food-habit data were collected only in the first few hours after sunset (e.g., Cockrum and Cross 1964; Jones 1965; O'Farrell and Bradley 1970). In the 1950s and 1960s, it was common to set mist nets over bodies of water, only to dismantle them shortly thereafter. Some studies at that time were specifically designed to evaluate food habits (e.g., Ross, 1967), and because these and similar efforts were frequently conducted by museum personnel, the animals were often prepared as museum specimens, which provided important information for ecomorphological studies.

During the past 20 years, stomach-content analysis has been replaced by fecal analysis as the preferred method for assessing diets of insectivorous bats (Kunz and Whitaker 1983; Rabinowitz and Tuttle 1982; Robertson and Stebbings 1993; Whitaker 1988). Analysis of culled parts is also a proven method, especially for gleaning bats that discard wings and head parts of prey at their feeding roosts (e.g., Bell 1982; Poulton 1929), but these studies were often biased toward large prey that bats carry to feeding roosts. The shift from stomach content to fecal analysis occurred largely in response to declining bat populations and restrictions imposed by state and federal laws protecting threatened and endangered species (Mohr 1972; Jones 1976) and for ethical reasons (Rudran and Kunz 1996). Identification of insect parts based on fecal analysis usually eliminates the need to sacrifice bats (Kunz and Whitaker 1983), although potential biases exist, especially if soft-bodied insects are digested and thus fail to show up in feces (Rabinowitz and Tuttle 1982; Robinson and Stebbings 1993).

Most published studies on diet, based on either stomach-content or fecal analysis, have ignored observations that many species have two or more feeding periods during the night (Lee and McCracken 2002; Whitaker et al. 1996). For example, a comparison of insect prey eaten by *Tadarida brasiliensis* in the pre-midnight period (mostly coleopterans) exhibited marked differences in composition as compared to insect prey taken in the pre-dawn period (mostly lepidopterans; Fig. 1). These observed differences by a single species within one night may be as great as or greater than differences reported for an entire assemblage of bats (e.g., Ross 1967; Whitaker 1972). Thus, failure to consider insects consumed by bats at different times of the night may severely bias conclusions about foraging behavior and dietary habits of a particular species. Moreover, failure to consider seasonal patterns of food intake can similarly bias conclusions about diets of insectivorous bats (Lee and McCracken 2002).

Variation in dietary habits can also be evaluated using stable isotope ratios of carbon (^{12}C : ^{13}C) and nitrogen (^{15}N : ^{14}N) (Hatch et al. 2002). Des Marais et al. (1980) compared stable isotope ratios of carbon and nitrogen in *Tadarida brasiliensis* and concluded that the

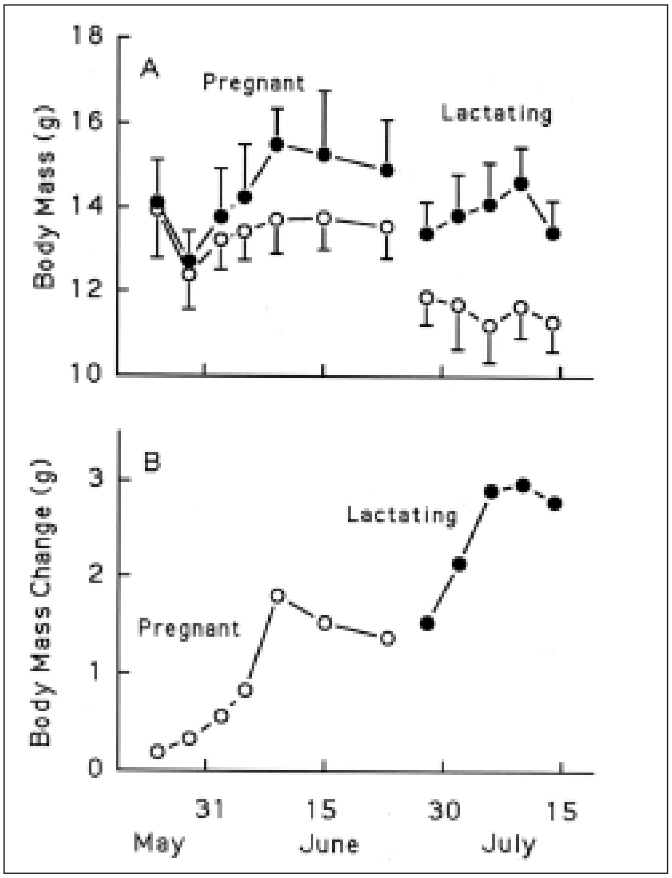

Figure 2: Variation in the quantity of food consumed by female Brazilian free-tailed bats (*Tadarida brasiliensis*) during the first feeding period during pregnancy and lactation (from Kunz et al. 1995).

diet of this species at Carlsbad Caverns in New Mexico included insects that had fed both on native vegetation (C3 plants) and on agricultural crops (e.g., maize, a C4 plant) in the region. This method also has been used to assess geographic variation in the diet *Leptonycteris curasoae* (Fleming 1995; Herrera et al. 1993).

The quantity of food that a bat consumes on a given night is of fundamental interest in ecologists, but few efforts have been made to quantify how much insectivorous bats actually eat. Such data have important implications for assessing ecological and economic impacts of bats on both natural and altered ecosystems. Estimates of dietary intake of selected North American insectivorous species have been based largely on gravimetric methods derived from field samples, estimates of feeding rates based on laboratory analyses, and from allometric equations (Nagy 2001; Speakman and Thomas 2003). For example, Gould's (1955) estimate of the amount of food eaten by *Eptesicus fuscus* was based on the mass of the stomach content of a single individual that was shot after it had foraged for an estimated 45 min. By extrapolating this mass to a one-hour feeding period, Gould calculated that the individual consumed 2.5 g of insects per hour.

Griffin (1958) assumed that *Myotis lucifugus* consumed 2 g of insects per hour, based on Gould's (1955) estimate of the mass of the stomach content from *M. lucifugus*. Kunz (1974) used a gravimetric approach to estimate nightly consumption in *M. velifer*, by weighing and comparing different cohorts of bats captured at hourly intervals, during the pre- and post-feeding periods. While these data were not used to estimate total nightly consumption, seasonal changes in estimated consumption from pregnancy through lactation suggested increasing levels of food intake as pregnancy and lactation progressed. Similarly, Anthony and Kunz (1977) and Kunz et al. (1995) used this method to evaluate patterns of nightly food intake during pregnancy and lactation in *M. lucifugus* and *Tadarida brasiliensis*, respectively (Fig. 2). Davis et al. (1962) used a gravimetric method to estimate nightly food intake in *T. brasiliensis* (1 g per night), but their estimate was considerably less than the nightly consumption estimated gravimetically for the same species by Kunz et al. (1995). Kunz (1980) published two equations for estimating nightly food consumption of insectivorous bats, one based on gravimetric changes in body mass and the other on the amount of feces produced. Estimates of digestive efficiency in insectivorous bats ranging from 75-78% (Barclay et al. 1992; Barclay and Brigham 1991) to 88% (Kunz, 1980) were then used to estimate consumption from fecal production.

Turnover of doubly labeled water (DLW) has been used to estimate nightly food intake in *Macrotis californicus* during the non-breeding season (Bell et al. 1986). Kurta et al. (1989) used this technique and estimated that lactating female *M. lucifugus* ingested the equivalent of 100% of a female's body mass (ca. 8 g) during peak lactation. As a first-order approach, estimates of nightly food intake can also be derived from allometric equations (Nagy 2001; Speakman and Thomas 2003), although these equations do not account for variation in season, sex, or reproductive condition.

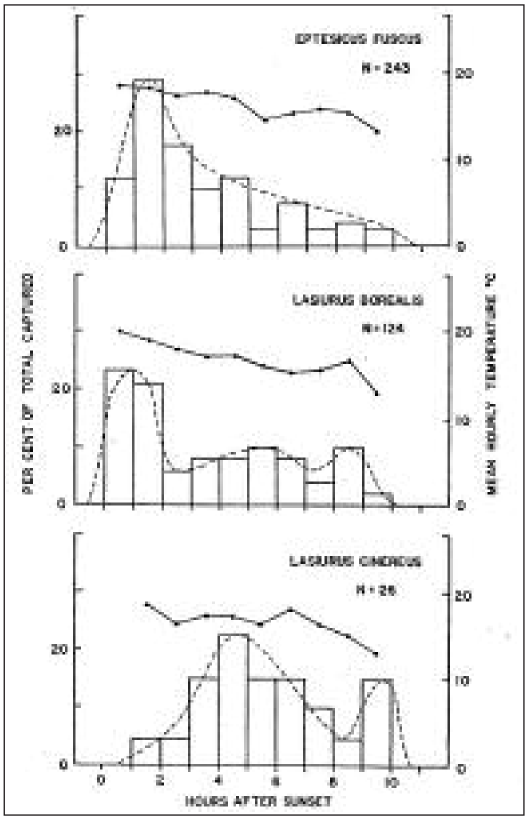

Figure 3: Temporal activity of selected species of bats captured in mist nets set over a small stream in central Iowa (from Kunz 1973). Mist nets were deployed from before sunset until after sunrise.

COMMUTING AND FORAGING BEHAVIOR

It has long been known that bats emerge from their roosts near dusk and return near sunrise. However, not until the 1960s were efforts made to document the activity of bats following the first foraging bout (e.g., Jones 1965). Few early studies sampled bats from dusk until dawn. In one of the first published studies in which netting was conducted throughout the night, Kunz (1973) demonstrated that several syntopic species showed peaks of activity at different times during the night (Fig. 3). Kunz (1993) recorded the number of bats captured at 15-min. intervals from dusk until dawn, over the course of three summers. These data provided the first convincing evidence that bats were active well past the first few hours after sunset, and that some species exhibit different patterns of activity. Direct captures not only make it possible to characterize the activity of each species, but also to determine sex, age, and reproductive condition (Kunz 1973).

One of the primary limitations of capture devices to assess commuting or foraging activity by bats is that capture success at a given location often decreases on subsequent nights following initial captures. Kunz and Brock (1975) reported that fewer *M. lucifugus* were captured in mist nets when they were deployed in the same location along a forest trail and over a small pond on subsequent nights (Fig 4). When nightly activity was monitored simultaneously with mist nets and heterodyne bat detectors (Holgate, Ltd) tuned to 45 kHz, the patterns of activity were similar (Kunz and Brock, 1975). Other studies, however, have reported contrasting patterns of activity based on mist nets and bat detectors (O'Farrell et al. 2000). In part, these observed differences in activity may reflect the fact that some species may be active above the height that mist nets can be deployed or, as noted above, bats may learn to avoid nets.

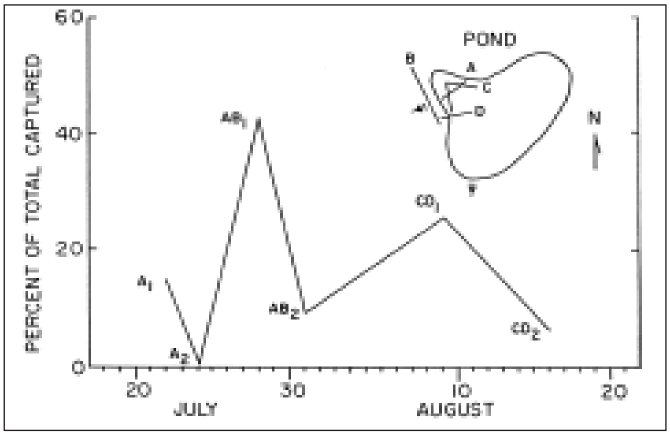

Figure 5: The effects of alternate mist net positions on capture success of bats. Net positions (lines) over and adjacent to the pond are designated by the letters (*A*-D). Short lines represent 6-m nets and long lines represent one 12-m or two 6-m nets. Positions and orientation are given for two ultrasonic detectors (net site and non-net site) at the pond. Letter combinations (with subscripts) denote the initial and subsequent nights that each net position was used (from Kunz and Brock 1975).

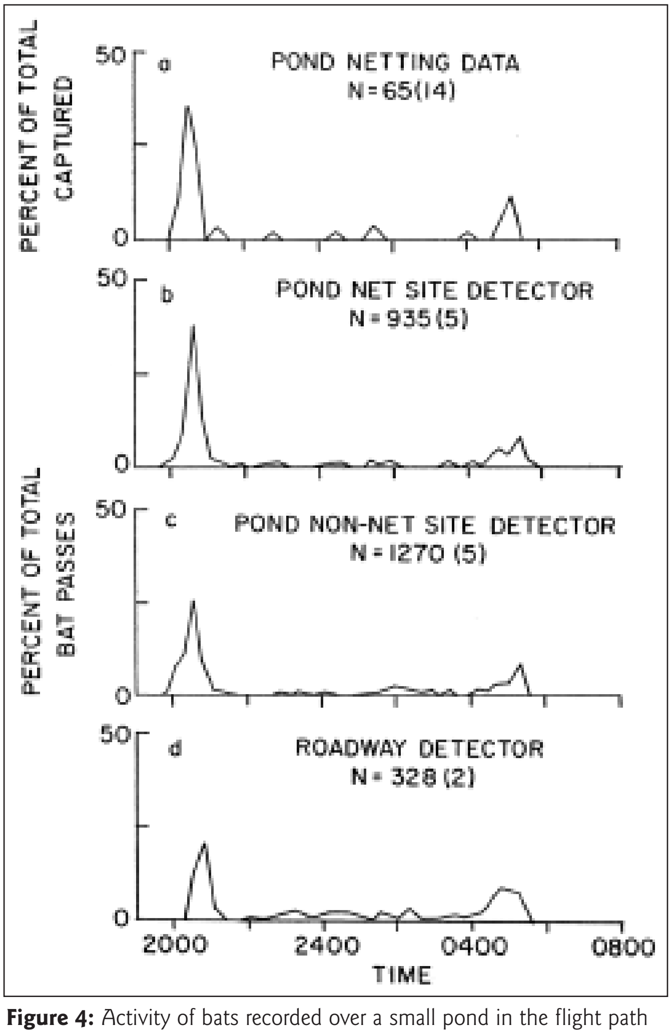

Figure 4: *A*ctivity of bats recorded over a small pond in the flight path of little brown myotis (*Myotis lucifugus*) in southern New Hampshire. The activity of bats is based on captures with mist nets and bat passes recorded with a bat detector (from Kunz and Brock 1975).

Deployment of canopy mist nets is one approach that has been used to assess the activity of bats as they forage or commute in the sub-canopy. These nets have been erected (most often several stacked nets) over streams, along trails, and in forest trails, although they

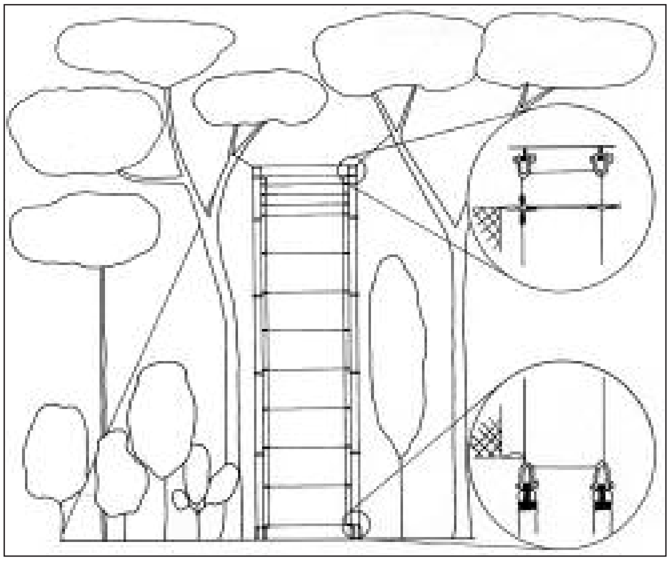

Figure 6: Rigging and position of a stacked canopy mist net extending from ground level to 30 m into the sub-canopy (from Hodgkison et al. 2002).

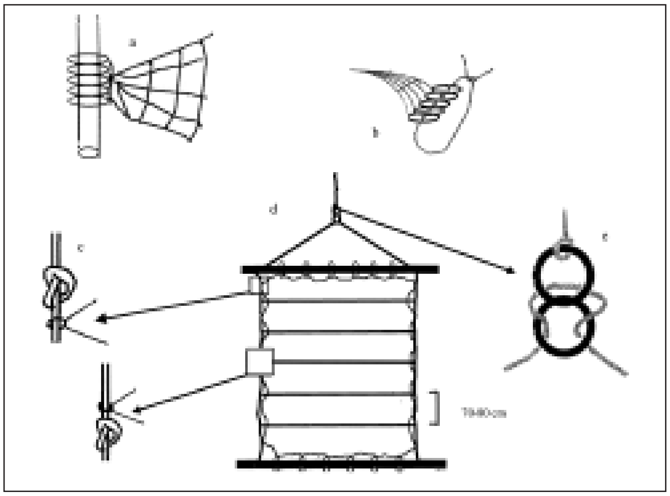

Figure 7: Preparation and deployment of an Avinet canopy mist net. **A:** Attachment of nylon loops to stationary object; **B:** Wire retaining loop holding the lateral, white plastic rings; **C:** Two knots in guidelines, flanking topmost plastic ring, with one knot in guideline placed below each subsequent ring; **D:** Frontal view of deployed canopy net; **E:** Closed S-hook assembly used to attach pulley rope and to balance net (from Rinehart and Kunz 2001).

require support from above and thus have limited application in open habitats. Handley (1967) was the first to report captures of tropical bats in the sub-canopy by deploying a series of stacked nets. Francis (1989), Ingle (1993), Kalko and Handley, (2001), and Hodgkison et al. (2002), have successfully used canopy nets to capture insectivorous and plant-visiting species in tropical regions (Fig. 6). Kurta (1982) used stacked nets positioned over streams to assess how bats used the space from the surface of the water to just beneath the overhanging riparian tree canopy.

Different configurations of canopy nets have been used. Most commonly, several standard 6- or 9-m-long mist nets have been stacked in a series and raised from ropes suspended from overhanging branches (reviewed in Hodgkison et al. 2002; Kunz 1988; Kunz et al. 1996). Munn (1991) described a method of reconfiguring standard mist nets by rotating the axis of the net 90 degrees and restringing the horizontal cords along the short axis of the net. This type of canopy net is now available commercially, although it requires special handling (Rinehart and Kunz 2001; Fig. 7). Alternatively, a series of up to 10 stacked nets requires specialized rigging to raise nets up to 30 m into the sub-canopy (Hodgkison et el. 2002). Macro mist nets have been deployed successfully in open areas to capture high-flying bats (Rautenbach, 1998).

RECORDING AND OBSERVING BATS IN DAY AND NIGHT ROOSTS

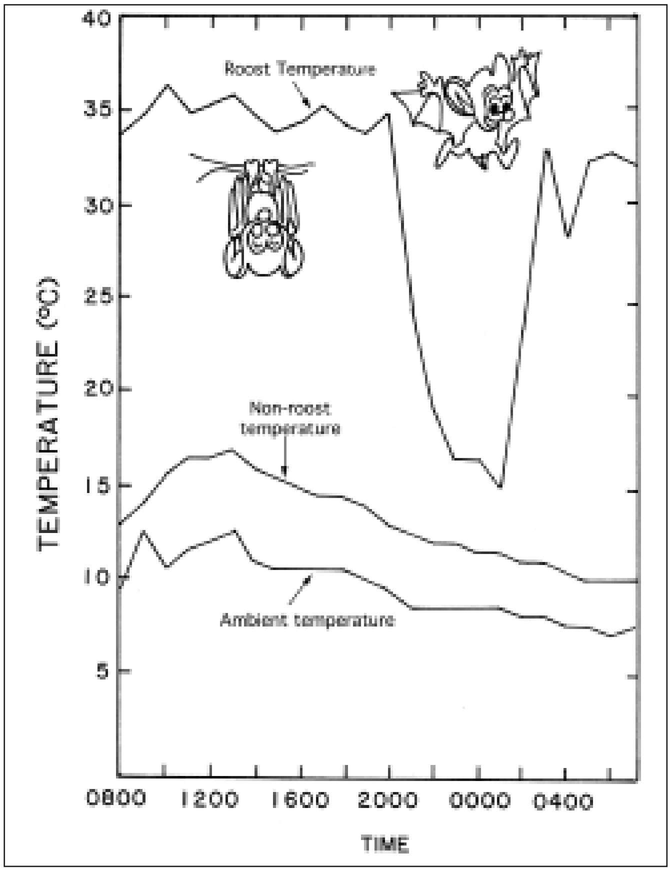

Figure 8: Temperature profile recorded in a maternity colony of cave myotis (*Myotis velifer*) occupying a crevice roost in a barn located in south-central Kansas (from Kunz 1974). The observed decrease in temperature in the maternity roost at sunset depicts the emergence from a roost crevice at the onset of the first foraging period of bats. Subsequent changes show a single night-roosting period and a second foraging period in the predawn period.

The timing of emergence, return, and commuting/foraging activity of bats can, in part, be determined by monitoring the day and night roosting activity, using changes in roost temperature or observations with night-vision devices or video recordings. Kunz (1974) used thermister probes coupled to a telethermometer to monitor day and night roosting activity of *Myotis velifer* in maternity roosts located in buildings. Thermisters were installed in roost crevices while bats were absent from the roost at night (Fig. 8). When bats occupied these roosts, temperature changes provided a direct measure of roosting activity and an indirect estimate of foraging activity. It was clear from

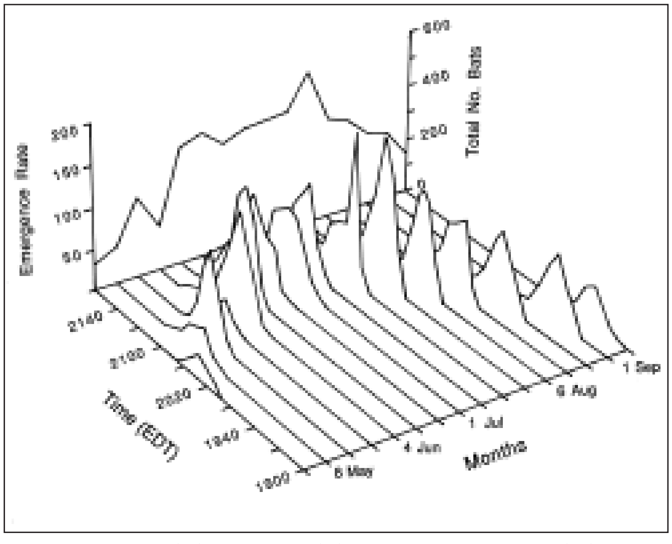

Figure 9: Seasonal changes in nightly emergence patterns of little brown myotis (*Myotis lucifugus*) from a barn located in southern New Hampshire (from Kunz and Anthony 1996).

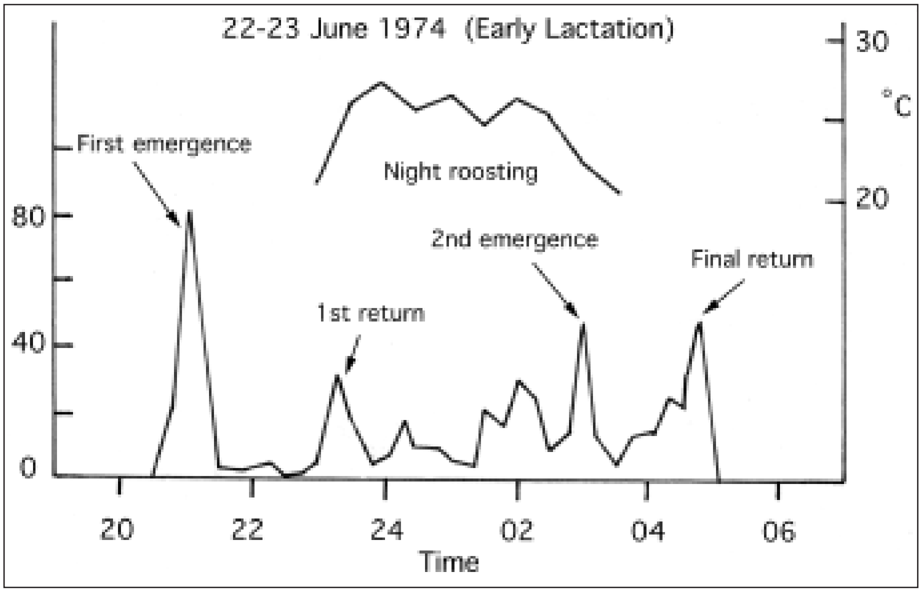

Figure 10: Nightly activity of little brown myotis (*Myotis lucifugus*) recorded with a bat detector (adjusted to 45 kHz) positioned in the pathway of bats as they emerged and returned to their roost (bat passes are designated on the left axis label). The initial activity reflects the peak of evening emergence, followed by a lull in activity (the first major feeding period). Subsequent activity indicates that some bats returned to occupy a night roost in the barn, followed by a secondary departure (depicting a second major foraging period). The predawn return is depicted as the large peak in activity shortly before sunrise. Changes in the temperature profile recorded in a night roost indicates when bats returned from feeding, the period of night-roost occupancy, and at times when bats departed for a second foraging bout (right axis label). The variation in temperature recorded during the night-roosting period corresponds to bat passes, indicating that there is some movement of bats in and out of the roost site throughout the night (T.H. Kunz unpublished).

these recordings that *M. velifer* had two synchronous departures during the maternity period (one after sunset and another after a period of night roosting and suckling of young). Similar methods were used to assess the nightly activity of *Pipistrellus subflavus* (Hoying and Kunz 1998). The assumption made from these records is that general activity patterns are a reflection of individual activity. With the availability of miniature, battery-operated temperature probes (e.g., Hobo Temperature Loggers), more detailed temperature recordings are possible.

Observations, using night-vision devices or infrared video cameras have also advanced our knowledge of daily and nightly roosting activity (Burnett and August 1981; Anthony et al. 1981; Winchell and Kunz 1996). By recording how much time bats occupy roosts, it is possible to estimate how much time they spend foraging (Kunz 1980). When data on roosting activity are used in combination with emergence counts (Kunz and Anthony 1996; Fig. 9), bat detectors deployed in the pathway of emerging and returning bats, and night roost temperatures (Fig. 10), it may be possible to estimate nightly time budgets (Kunz 1980).

Data on patterns of nightly activity derived using these different methods are estimates of average time budgets, but they provide no data about individual activity. Assessing the activity of bats using temperature telemetry (Kunz, 1974) is generally limited to bats that roost gregariously.

Nightly Activity Based on Capture with Harp Traps

Harp traps are more effective for collecting data on temporal activity than mist nets in many situations, largely because they make it possible to handle large numbers of bats without the necessity of removing individuals from nets. Kunz (1974) used a two-frame harp trap (Tuttle 1974) set in the open doorway of barns and caves that housed maternity colonies of *Myotis velifer* in south-central Kansas. The nightly activity patterns of captured bats illustrate seasonal changes in female (Fig. 11A) and juvenile activity (Fig. 11B). If traps are monitored at more frequent intervals, high-resolution data on activity is possible. In these situations, harp traps should not be placed so that they cover the entire opening used by bats. Notwithstanding, some bats learn to avoid being captured, bounce off, or sometimes pass through the trap (Kunz and Anthony 1977), thus potentially contributing to biased results.

Observing Commuting and Foraging Bats

Results Using Light Tags

Buchler (1976; 1980) used light tags to assess dispersal, commuting, and foraging behavior of insectivorous bats. Others (reviewed in Barclay and Bell 1988) have also used this technique, with the greatest success rates recorded in open areas, along flyways, and adjacent to forest edges. Buchler and Childs (1981) used light tags on *E. fuscus* and concluded that this species dispersed nightly to feeding grounds by following cues produced by calling frogs and stridulating insects. Light tags have also been used successfully, in combination with bat detectors, to follow known individuals while their echolocation calls are monitored. The main limiting factor of using light tags in heavily forested areas is that the bats often quickly disappear from view. They are most successful in open habitats.

Results from Radiotelemetry

Williams and Williams (1967; 1970) were among the first to use radiotelemetry with bats, specifically to assess homing behavior in *Phyllostomas hastatus*, a Neotropical omnivore. Early radiotelemetry studies were limited to relatively large species, because the components and batteries at the time were relatively large and bulky compared those presently available (< 0.4 g). With increased miniaturization of transmitter components and the availability of small, relatively long-lasting batteries, radiotelemetry has become a standard method to assess the foraging habits and roosting behavior of relatively small (>8 g) species (e.g., Barclay 1989; Barclay and

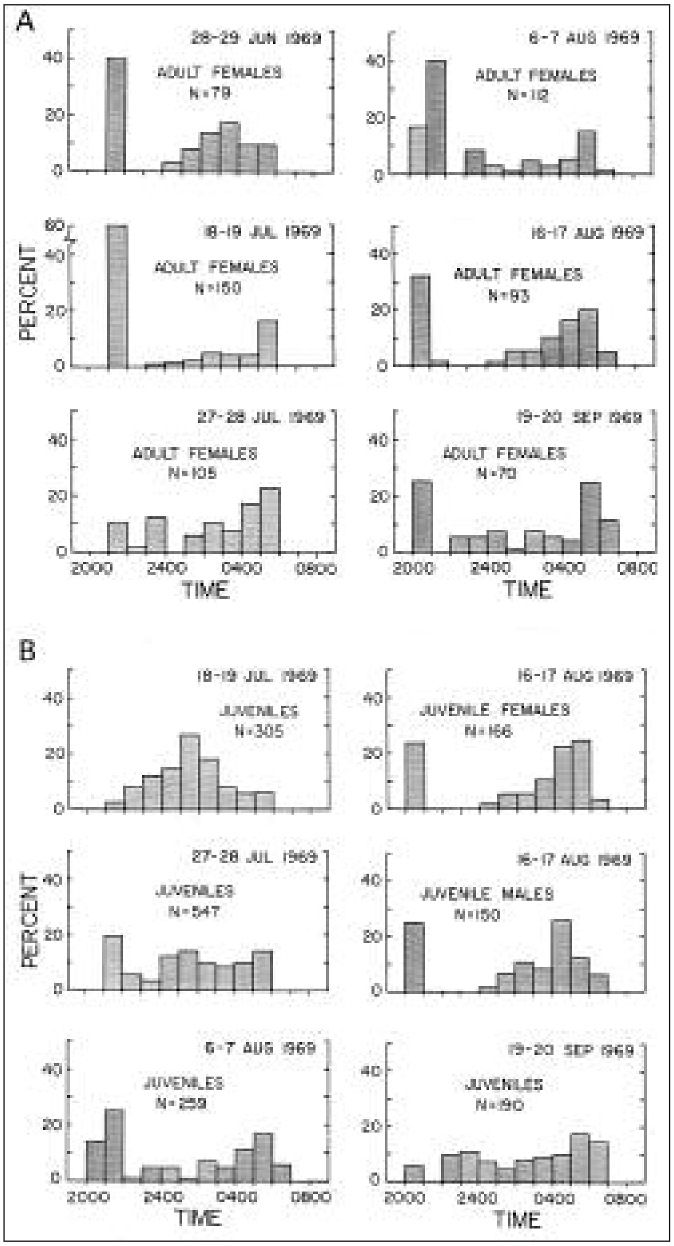

Figure 11A: Nightly flight activity of adult female, and **B:** juvenile cave myotis (*Myotis velifer*) captured with a two frame harp trap placed in the open doorway of a barn in south-central Kansas (after Kunz 1974).

Brigham 1991; Clark et al. 1993, Fuhrmann and Seitz 1992; Gumbert et al. 2002; Jones and Morton 1992; Jones et al. 1995; Krull et al. 1991; Kunz et al. 1998). Radio transmitters that include mercury switches make it possible to determine when bats are flying or roosting. In some cases, it is possible to determine activity based on the modulated signals when bats are in flight. With access to computers, GPS units, data loggers, and specialized software, radiotelemetry can be used to infer roosting habits, home range, foraging behavior, and time budgets of individual bats.

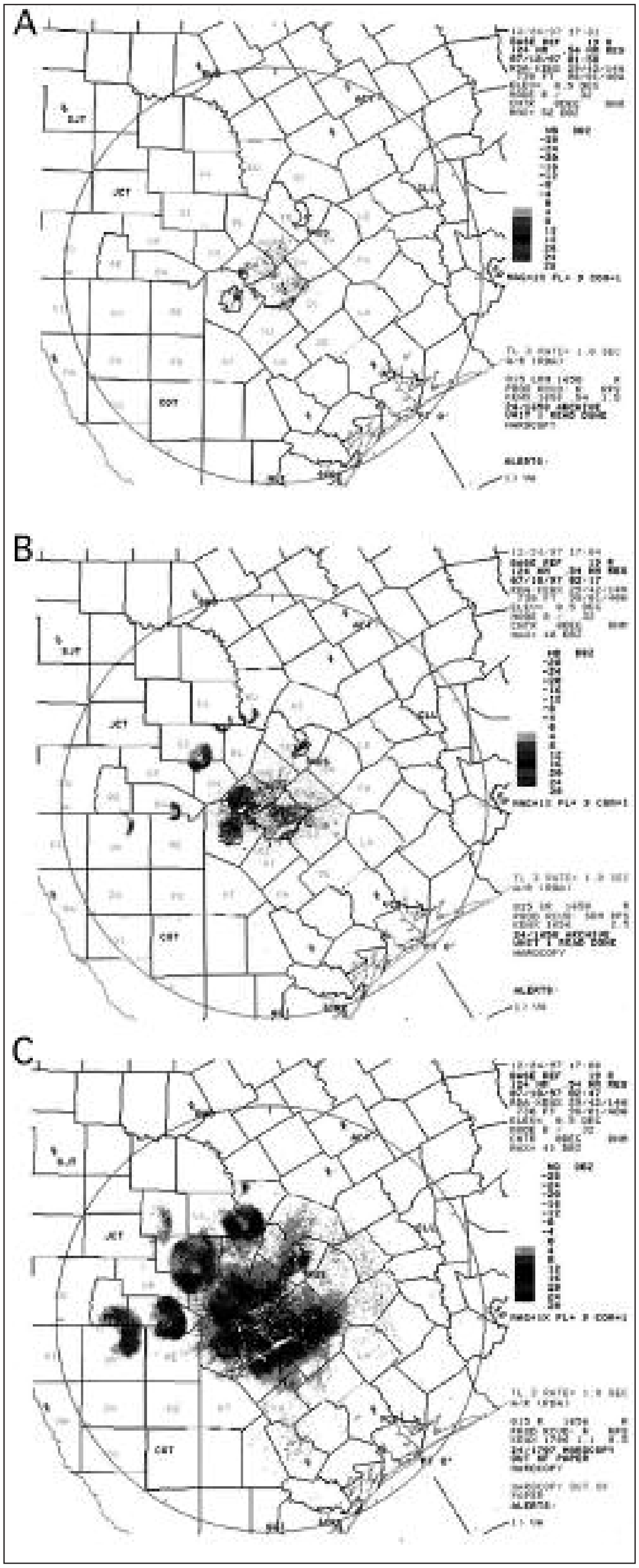

Figure 12: Doppler radar images of Brazilian free-tailed bats (*Tadarida brasiliensis*) dispersing from a maternity roost in south-central Texas. The first image (**A**) shows the radar reflectivity shortly after the first emergence. The second and third images show the changing patterns of dispersal 15 and 30 minutes later, respectively (T.H. Kunz unpublished).

Field Observations and Recordings of Echolocation Calls

Based on field observations, several investigators have classified bats into different foraging strategies (e.g., Kalko and Schnitzler 1998; Neuweiler 1989; Norberg and Rayner 1987). These foraging strategies are based on unique echolocation signals and the relative amount of physical clutter present (Kalko and Schnitzler 1998). Making direct observations of flying bats in concert with recording echolocation calls is essential for a full understanding of foraging (Limpens this volume).

Recordings of echolocation calls synchronized with multi-flash, 3-dimensional photography or videography can provide valuable data on the behavior and echolocation-call structure associated with prey capture (Kalko, this volume). Multi-flash photographic sequences of aerial captures of insects by echolocating bats suggest a shift from a search mode to a target-oriented approach, immediately after bats detect potential prey (Kalko and Schnitzler 1998). Multi-flash recordings in open habitats indicate that bats often approach their prey from behind, moving into optimal positions immediately above the insect being pursued. In contrast, gleaning bats usually fly directly towards their prey, making captures by pouncing on the prey (Kalko and Schnitzler 1998).

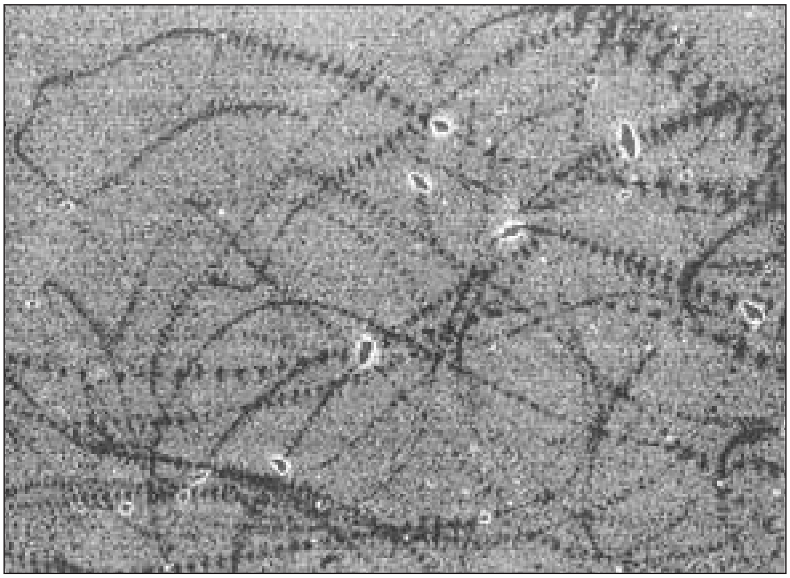

Figure 13: Infrared thermal image of Brazilian free-tailed bats (*Tadarida brasiliensis*) foraging over an open field. Trajectories of bats are depicted by trailing wing prints (T.H. Kunz and J. Frank unpublished).

FROM ECHOLOCATION TO FORAGING

Captive Studies

Much of what has been learned about the foraging habits of insectivorous bats has come from laboratory studies of echolocation and prey capture. In his classic book, *Listening in the Dark*, Griffin (1958) eloquently summarized what had been learned from recordings of echolocation calls in the laboratory during the early period of discovery (reviewed in Griffin, this volume). In the intervening years, enormous progress has been made, especially in the development and use of bat detectors for recording echolocation calls and the refinement of specialized software for analysis (Corben, this volume; Pettersson, this volume; Szewczak, this volume). Some of the most promising research on foraging by insectivorous bats has combined studies of behavior in captivity with observations in the field (see Jones, this volume; Seimers, this volume; Kalko, this volume). Future studies on foraging habits that combine laboratory and field-based research on echolocation promise to provide revealing insights with respect to how bats actually navigate and forage in different environments.

Habitat Use Based on Bat Detector Surveys

One of the current applications of bat detectors is to assess the composition of local assemblages and characterize nightly activity patterns. To accomplish these goals requires reliable species identification. Unfortunately, many efforts to date have been hampered by inappropriate sample design and uncertainties about species identity (e.g., Hayes 1997; Hayes 2000). Until these uncertainties and protocols can be addressed, bat detectors for general surveys will provide little useful information with respect to how and when insectivorous bats commute and forage in different habitats.

Direct Observations

In some instances, it may be possible to visually observe bats as they forage in open habitats, especially near sunset and at high latitudes with extended period of twilight (e.g., Nyholm 1965; Rydell 1986). At most other times and places, however, opportunities to directly observe and record the foraging behavior of insectivorous bats requires artificial illumination (Rydell and Racey 1995).

ADVANCED IMAGING TECHNOLOGIES

Doppler Radar

Where NEXRAD Doppler radar installations exist, this technology can be used to quantify nightly behavior of bats as they disperse and forage. For example, the Brazilian free-tailed bat (*Tadarida brasiliensis*) in the southwestern United States forms enormous colonies that often disperse upwards of 70 km from their daily refuges. These movements can be monitored by recording the reflectivity of the dispersing animals using NEXRAD Doppler radar (Fig. 12). These data are available from the U.S. government and some private organizations and can be downloaded for quantitative analysis of time, direction, and density of dispersing bats (J. Horn and T.H. Kunz, unpublished). This technology offers a powerful tool for quantifying the nightly dispersal and foraging activity of species that form large colonies, which is not otherwise possible.

Infrared Thermal Imaging

Infrared thermal-imaging cameras also offer opportunities to record the nightly emergence and feeding behavior of bats independent of natural or artificial illumination. Infrared thermal cameras are designed to detect heat emitted by objects as long as they are warmer than the background. Coupled to computers, infrared thermal images has been used successfully to census bats as emerge from roost sites (Frank et al. 2003; Sabol and Hudson 1995) and to document flight trajectories during foraging bouts (Fig. 13).

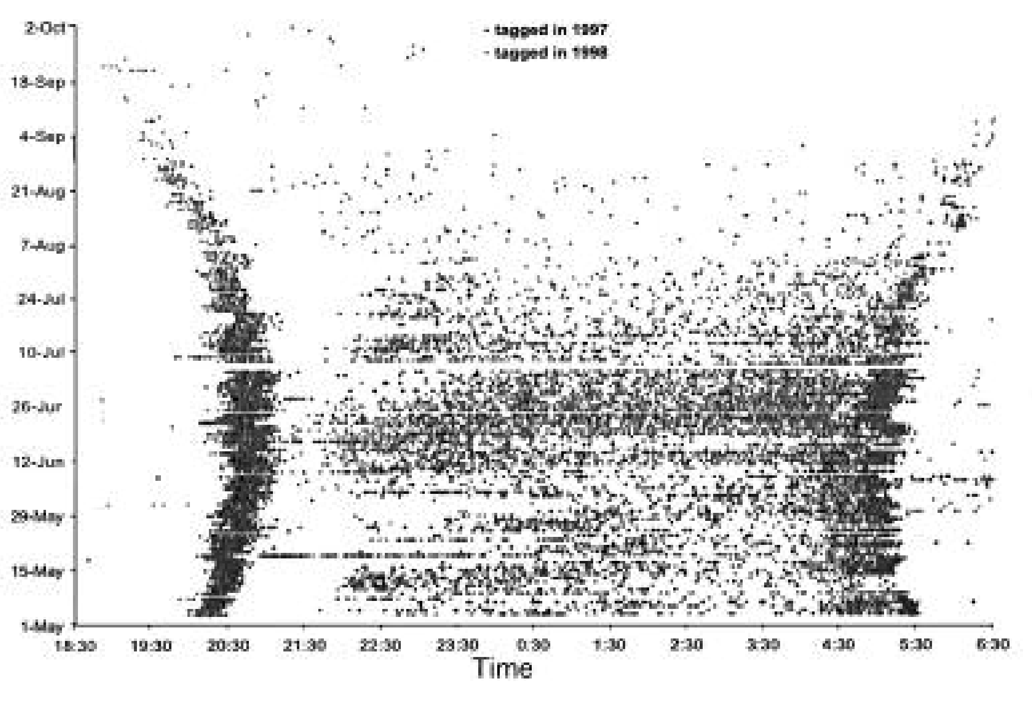

Figure 14: Nightly activity of the little brown myotis (*Myotis lucifugus*) recorded with passive integrated transponders (PIT tags) as individuals departed and returned from a barn in southern New Hampshire. Bats must pass through a loop antenna to be recorded. The pregnancy and lactation periods are shown for comparison. The records for juvenile bats, when they first began to depart from the barn to forage are depicted as lighter colored dots (J. Horn and T.H. Kunz unpublished).

Passive Integrated Transponders (PIT tags)

Only a few studies have used passive integrated transponders (PIT tags) to investigate the ecology and behavior of bats (Brooke 1997; Kerth 1996). PIT tags offer an excellent opportunity to record roosting habits and, by absence, to infer foraging by bats. Recently, PIT tags have been used to investigate the emergence and return times and use of night roosts at a maternity roost of *Myotis lucifugus* (J. Horn and T.H. Kunz, unpublished). When bats pass through a loop antenna positioned strategically over an opening used for exit and return or through cavities used as night roosts, it is possible to record the activity of individuals. When data for an entire season are plotted, patterns related to season (day length), reproductive condition, and age can be obtained (Fig. 14). From these data, it is possible to quantify time budgets of individuals.

ECOMORPHOLOGY

The structure of wings, skull, and dentition of bats provides a valuable means to predict foraging habits (e.g., Freeman 1998; Norberg 1998; Norberg and Rayner 1987; Stockwell 2001; Swartz 1988; Swartz et al. 2003). Wing shape, wing loading, and aspect ratio are three important variables used to compare and predict the foraging habits of bats in different environments (e.g., Adams 1998; Aldridge and Rautenbach 1987; Barclay and Brigham 1991; McKenzie et al. 1995). Wing structure of bats is often highly correlated with echolocation-call design, flight mode, speed, and the amount and type of environmental clutter present (e.g., Aldridge and Rautenbach 1987; Heller and von Helversen 1989; Jones et al 1993; Kingston et al. 2000).

Wing structure has also been used to evaluate intraspecific differences in foraging behavior (e.g., Adams 1997; Adams 2000). For example, Adams (1996; Adams 1997; Adams 2000) found that young *Myotis lucifugus* typically forage in less cluttered environments when they first begin to fly, but shift to more cluttered habitats as they perfect foraging skills and wing dimensions reached adult size. Differences in wing structure may also differ geographically within a given species, especially in habitats where the species assemblage may differ. Developments in computer simulation (Stockwell 2001), computer modeling of wing design, and studies that take into consideration the dynamics of wing shape and function during flight promise to provide more realistic interpretations of foraging behavior than is possible using static models (Swartz et al. 2003).

The predictive power of ecomorphological studies can guide field workers evaluating diet, and field-based biologists who study diets of bats can provide research direction to ecomorphologists. The skull and dentition, in particular the shape and size of teeth, are important structures that can be used to interpret and predict the diets and trophic status of different species (e.g., Freeman 1979; Freeman 1992; Swartz et al. 2003). The nature of food items and how the teeth of insectivorous bats have evolved has been the subject of considerable research (Freeman 1988; Freeman 1998). For example, consumers of hard-bodied prey can often be distinguished from those who eat soft-bodied prey by their more robust mandibles and crania, larger but fewer teeth, longer canines, and abbreviated third upper molars.

CONCLUSIONS

Biologists interested in studying the foraging habits of insectivorous bats have an increasing array of devices and analytical methods at their disposal. These include traditional tools, such as mist nets and harp traps for capture, and also specialized instrumentation for recording and analyzing echolocation calls. They also include instruments and analytical methods for quantifying movements of animals during nightly dispersal and while foraging in different habitats, assessing diet composition, quantifying nightly food intake, and evaluating the function of structures (wings, crania, and teeth). Depending on the scope of an intended study, the particular species or species assemblage being investigated, and the specific questions being asked, complementary approaches should be employed, both in the field and in the laboratory.

ACKNOWLEDGMENTS

I wish to thank several of my current and former students (Edythe Anthony, Peter August, Carol Brock, Christopher Burnett, Jason Horn, Karen Hoying, Tigga Kingston, Allen Kurta, Simon Robson, Tim Rumage, and Mike Wadanoli) and several colleagues (Robert Barclay, Ed Buchler, Brock Fenton, Gary McCracken, Ken Nagy, Ulla Norberg, Merlin Tuttle, and John Whitaker) whose suggestions and advice have helped guide many of the ideas summarized in this paper, although I take full credit for any misrepresentation of fact or interpretation. My research on insectivorous bats in North America has largely been supported by grants from the National Science Foundation.

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Section 2

ACOUSTIC INVENTORIES

ULTRASOUND DETECTION: BASIC CONCEPTS

CHOOSING A BAT DETECTOR: THEORETICAL AND PRACTICAL ASPECTS

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We emphasize that there is no "best" bat detector. Each of the three basic types of detector systems available today – heterodyne, frequency division, and time expansion – has different capabilities and limitations. We review these capabilities and limitations within the context of the goals of different research and/or monitoring projects, the bat fauna being investigated, and our own experience. We summarize the practical and technical features of different detector systems in relation to 1) sensitivity, 2) ability to record and process ultrasound, and 3) ability to describe the parameters necessary for the identification of bats to species. We also categorize a range of possible applications of detector types to field research with regard to scale and resolution in space and time. On the basis of the relationship between research questions and the resulting research design, we discuss the choice of the most appropriate detector or combination of detector systems for general and specific applications.

Key words: bat detectors, habitat monitoring, practical considerations, species identification, technical details
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ULTRASOUND CONVERSION SYSTEMS

Here we briefly describe the features of different ultrasound detection and conversion systems. For detailed explanations of the technical functioning and properties of the different systems, see Ahlén et al. (1984), Fenton (1988), Parsons et al. (2000), Pettersson (1993a, 1993b, 1999, this volume). The time-expansion (TE) detector system samples and digitizes the signal and plays it back over an expanded time. TE detectors are broadband systems that detect the entire frequency band used by bats, but the 'slowed-down' output is no longer in real time. The time-expansion factor can be of any size, but factors of 10 or 20 are most commonly used. As with a tape recording played back slower than the recording speed, frequencies in the output of a TE detector are lower and the pulses longer depending on the expansion factor (Fig. 1). Using a time-expansion factor of 10, a typical frequency-modulated (FM) pulse sweeping from 70 to 25 kHz in 5 ms will be played back as an audible signal sweeping from 7 to 2.5 kHz in 50 ms. Provided sampling has been arranged correctly in the detector design, the physical properties of the signal are preserved and the output is excellent for sound analysis. Many details of time-expanded signals also can be interpreted by ear in the field.

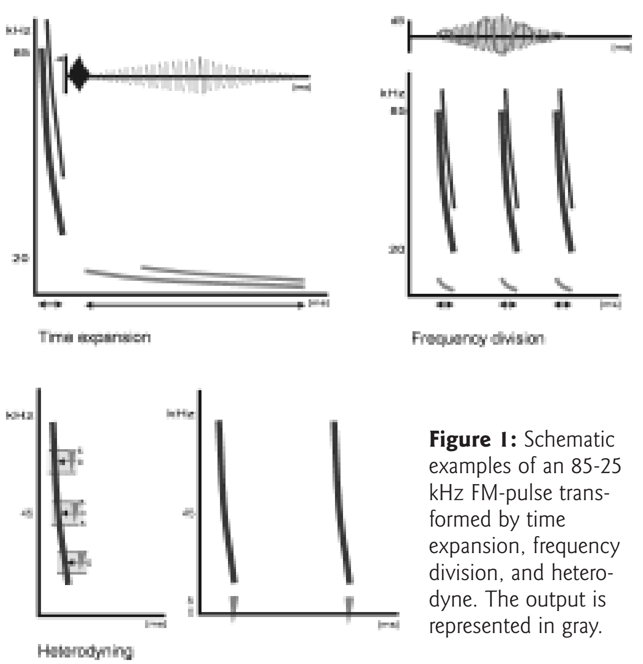
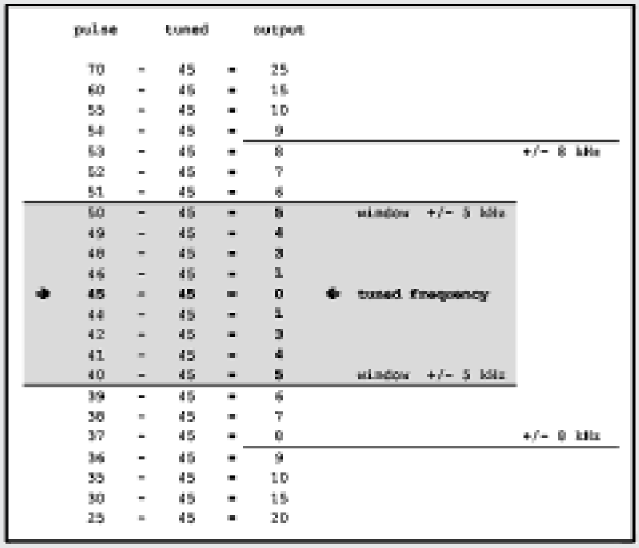


Figure 1: Schematic examples of an 85-25 kHz FM-pulse transformed by time expansion, frequency division, and heterodyne. The output is represented in gray.

A major limitation of this system is that TE detectors sample intervals of time, as determined by the detector's storage capacity, but they do not monitor incoming calls while in playback mode. Therefore, TE detectors cannot continuously monitor bat activity. The frequency-division (FD) detector is also a broadband system that monitors the entire frequency band in which bat signals might be detected. The FD system counts the waves (cycles) of the incoming signal by counting the time between the zero crossings and produces a single wave for a set number (e.g., 10) of waves counted. The division factor can be of any size, but 'divide by 10' is commonly used. At 'divide by 10', an FM pulse sweeping from 70 to 25 kHz in 5 ms will generate an audible output sweeping from 7 to 2.5 kHz in 5 ms, which can be recorded and interpreted in real time (Ahlén et al. 1984; Andersen and Miller 1997; Pettersson 1993a, 1999; Fig 1). Because the output is in real time, the frequency-division system can



	pulse		tuned		output	
	70	-	45	=	25	
	60	-	45	=	15	
	55	-	45	=	10	
	54	-	45	=	9	
	53	-	45	=	8	+/- 8 kHz
	52	-	45	=	7	
	51	-	45	=	6	
	50	-	45	=	5	window +/- 5 kHz
	49	-	45	=	4	
	48	-	45	=	3	
	46	-	45	=	1	
◆	**45**	-	**45**	=	**0**	◆ tuned frequency
	44	-	45	=	1	
	42	-	45	=	3	
	41	-	45	=	4	
	40	-	45	=	5	window +/- 5 kHz
	39	-	45	=	6	
	38	-	45	=	7	
	37	-	45	=	8	+/- 8 kHz
	36	-	45	=	9	
	35	-	45	=	10	
	30	-	45	=	15	
	25	-	45	=	20	

Table 1: An example of input and output frequencies when heterodyned or mixed with an oscillator signal (i.e., the tuned frequency) of 45 kHz. In different detectors, low pass filters may be broader or narrower, resulting in a broader or narrower frequency window.

be used to continuously monitor the activity of bats.

Limitations result from the process of counting the time between the zero crossings because the sinusoidal waveform is converted to a square wave in the output signal, thus losing some of the physical information in the call. Also, the FD detector converts zero crossings for only the most intense frequency and does not provide information on harmonics. The process of 'averaging' groups of 10 waves also makes it impossible to evaluate rapid frequency changes. These typically occur at the beginning or end of a pulse where rates of frequency change tend to be relatively high in FM signals of bats. In that respect, FD detectors give less reliable measures of start and end frequencies in the steep FM parts of pulses.

The signal produced by FD detectors can be used for sound analysis, but physical detail in the signal is lower than from TE detectors. In some systems, the pulse envelope is determined and superimposed on the output wave. In this way, information on amplitude is preserved. A Fast Fourier Transform analysis of a FD signal, e.g. to calculate peak frequency, will however be much less accurate than in a TE signal. A zero-crossing analysis, through its nature, would be more accurate, but as a consequence of the averaging of groups of 10 cycles is still not as accurate as in TE.

Heterodyne detectors use a variable-frequency oscillator and mix the incoming frequencies of the bat(f_{bat}) with the oscillator frequency tuned in the detector(f_{tuned}). The resulting signal contains the sum frequencies ($f_{tuned} + f_{bat}$) as well as the difference frequencies ($f_{tuned} - f_{bat}$) as frequencies with high energy. The sum frequency will be higher than the ultrasonic frequency of the bat and will be filtered out. Where the frequency of the pulse is near the tuned frequency, the difference frequency will be low, reaching 0 kHz where $f_{tuned} = f_{bat}$. As an example, with a setting of the oscillator frequency at 45 kHz, an FM pulse sweeping from 70 kHz to 25 kHz would produce a V-shaped output sweeping from 25 to 0 kHz and up again to 20 kHz. A low-pass filter in the output (e.g., 5 or 8 kHz) functions as a 'frequency window,' filtering out higher frequencies and providing a clear, audible signal, sweeping down from 5 to 0 kHz and up to 5 kHz again (Table 1, Fig. 1). The same output, however, will be produced at detector settings of 55 or 35 kHz, and there is no frequency information available from the output signal (Fig. 1). The frequency at which the bat detector is tuned provides the only information on the frequency of the incoming signal.

REQUIREMENTS OF A DETECTOR

The requirements that we place on detectors for detecting bats closely resemble the requirements of a bat trying to detect insect prey. As for a bat hunting an insect, we wish to detect the presence of a bat and, with a certain accuracy and resolution, know its position (distance and angle) and possible flight course. In this context. we discuss a number of different requirements and features.

Detection Range and Sensitivity

Ideally, the range over which a bat is detected should be as large as possible. This allows the sampled area to be larger and the number of observations higher. Greater range allows for an earlier detection of bats resulting in a longer observation time, which enhances recording and identification possibilities.

Detection range depends on several variables that include the frequency range and strength (amplitude) of the emitted signal, the sensitivity of the microphone to different frequencies, the orientation of the bat to the microphone, whether the bat is flying in open or cluttered airspace, and atmospheric conditions. The amplitude of calls and the signal type used by the species of bat (Fig. 2) are dependent on habitat and the distance a bat is from surrounding obstacles that produce clutter (i.e., echoes from objects other than the target,

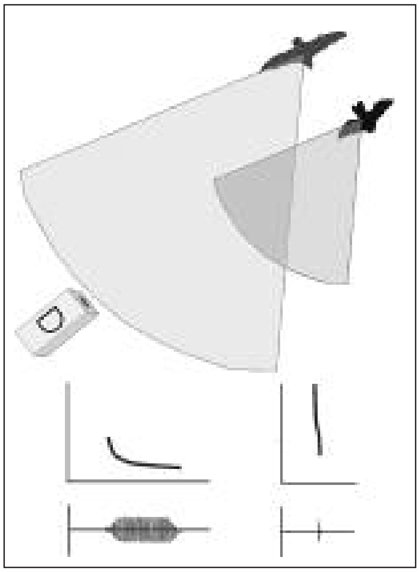


Figure 2: The range over which a bat is detected is dependent on call intensity and the type of signal (frequency, bandwidth) used by the bat.

Fig. 3). For example, *Nyctalus noctula* hunting high in open air, using frequency-modulated, quasi-constant-frequency (FM-QCF) pulses, will be detected at a greater range than *Eptesicus serotinus*, which also hunts in the open, but closer to clutter using somewhat shorter FM-QCF pulses. In general, bats using QCF or shallow FM calls are louder then those using strictly FM calls. Clutter also influences the calls used by bats. Bats hunting in clutter or hunting closer to vegetation will use steeper pulses, which are detected over a shorter range. Higher frequencies in the more intense QCF part of the pulse also have a lower detection range. The calls of *Pipistrellus pygmaeus* (QCF 55 kHz) have a similar amplitude as those of *P. pipistrellus* (QCF 45 kHz; Holderied 2001), but are detected over shorter distances. These factors result in species- and habitat-specific differences in the sampling areas of detectors and affect the probability of detecting different species in different habitats (Fig. 3).

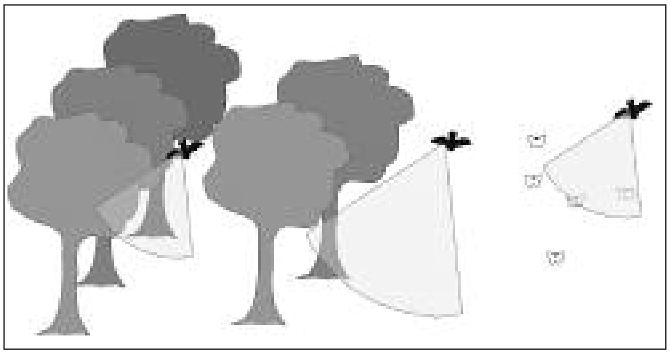


Figure 3: Echolocation calls are adapted to the habitat or amount of clutter, resulting in different calls for the same species in different habitats.

Independent of the kind of system, the detector should be functioning properly, the microphone should be kept dry, microphone sensitivity and/or gain should be set correctly, and the batteries should be charged. Battery condition is less of a problem in detectors designed with internal voltage stabilizers, which keep the detector's sensitivity stable until a threshold battery voltage is reached. Capacitance microphones are more sensitive than electret microphones to factors such as high relative humidity, low temperatures, and strong wind. Electret microphones are almost exclusively the ones used in heterodyne and FD systems. Exceptions include the Ultrasound Advice S25 FD detector, which uses a capacitance microphone. TE systems use either capacitance or electret microphones, depending on cost and application for detecting higher-frequency calls.

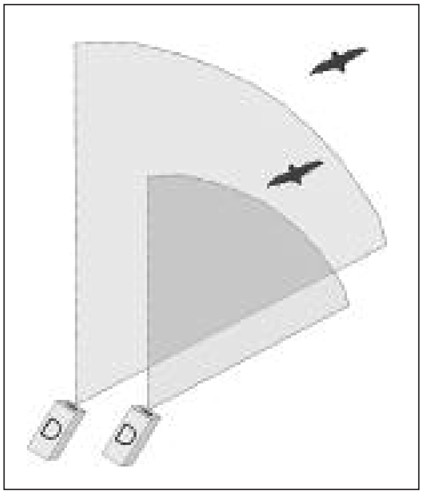


Figure 4: Detection range differs according to the sensitivity of the detector system.

Sensitivity differs between detector systems (Fig. 4) due to various technical properties. These properties include transformation of the entire (TE, FD) or part of the frequency range (HET), low-pass filtering of noise (HET), the use of threshold-sensitive circuits (FD), differences in frequency response, and overall sensitivity of types of microphones. In practice, heterodyne detectors are highly sensitive (Parsons 1996; Waters and Walsh 1994), and detection range is enhanced by the ability to tune to the most intense part of the signal (i.e., best listening frequencies or the loudest QCF frequencies, Table 2). This is an important advantage for detecting quiet-echolocating species such as *Myotis*, *Plecotus*, and many tropical Phyllostomid species. Time expansion is the next most sensitive method, but detection range is less than in heterodyne detectors. Frequency-division detectors, using threshold-sensitive circuits are the least sensitive with the most limited range (Table 2).

Directionality of the Microphone

Directionality is a quality of the microphone rather than of the detector system. There are tradeoffs regarding the microphone. A more directional microphone provides better indication of the bat's position in the habitat and improves the potential for visually observing flight behavior. High directionality also allows observers to concentrate on a part of habitat (e.g., bats passing on flight path, bats above water surface). Directional hand-held devices make it possible for observers to scan the surroundings, whereas a mounted directional microphone in a stationary setup will be selective for the part of the surroundings or habitat at which the microphone is pointed.

A less directional microphone enlarges the sampled area, and enhances the chances of detecting a bat. There is a trade-off between directionality and range. The less directional, the shorter the detection range (i.e., sensitivity), which reduces the chance of detection (Fig. 5).

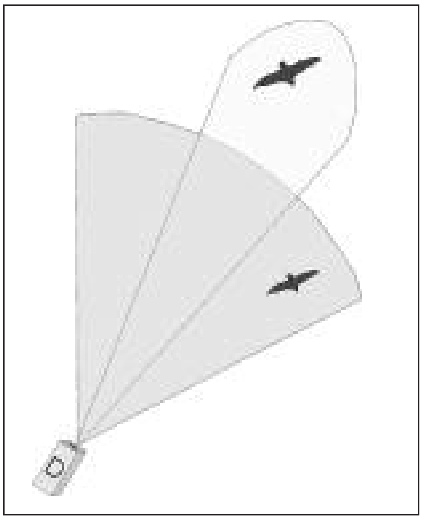


Figure 5: Directionality of the microphone allows for a better location of a bat in the surrounding habitat. There is a trade-off between range, angle, and directionality resulting in different probabilities of detecting a bat.

	auditive (in field)			analysis (in field or lab)		
	heterodyning system	division system	time expansion system	heterodyning system	division system	time expansion system
detection range	++	+/-	++			
monitored bandwidth	+	++	++		++	++
tuning/focusing on frequencies	++	-	-		++	++
frequency related parameters						
tonal quality: presence/absence	++	+/-	+			
tonal quality: pitch	++	+/-	++			
FM-rate / shape / curvature	-*	-*	++		+	++
Doppler effect	++	-	-			
alternation of pulse types	++	-	+		+	++
best listening frequency	++	-	-		+	++
QCF frequency	++	-	-		+	++
distribution of energy	++	-	+/-		+	++
presence of harmonics	++	-	-		-	++
frequency range / bandwidth	+	-	-		+	++
F_{max}	-/+	-	-		-/+	-/+
F_{min}	+	-	-		+	++
amplitude related parameters						
loudness	++	++	++		+	++
distribution of energy	++	-	+/-		+	++
time related parameters						
pulse length	+/-	+/-	++	-	+	++
interval length	++	++	+/-	++	++	++
repetition rate	++	++	+/-	++	++	++
rhythm	++	+	+/-	?	?	?
social sounds	+	+/-	++	-	+	++
price level	++	+	+/-			

Table 2: A comparison of heterodyne, frequency-division, and time-expansion detection and conversion systems with respect to the potential these systems provide for interpretation of audible parameters for identification in the field and/or the analysis of recorded sound involving oscillograms, multi-spectrograms, frequency spectrograms, and zero crossing.

Legend: ++ = good, + = possible, +/- = difficult, - = not possible,
or: ++ = relatively inexpensive, + = intermediate, +/- = relatively expensive.
* no direct information, but information on FR rate/shape/curvature can be deduced from tonal quality, change of pitch while tuning, position of QCF, or shallow FM in the pulse relative to steeper FM.

The chance of detection also depends on the nature of the habitat and amount of clutter present. In an open field, any system will sample a cone-shaped volume with a relatively even detection probability within this cone. At a forest edge or on a forest path, the shape of the cone will be biased towards the open part of the habitat (Fig. 6). The directionality of a microphone is also frequency dependent, with more directionality at higher frequencies than at lower frequencies. In wide-angle microphones, frequency will have more impact than in directional microphones. So at least theoretically, we may expect differences in sampled area, and in detection chance between species in relation to the frequencies they use. The sound produced by the bats also varies in its directionality.

In studies whose purpose is to make detailed observations of flight course and flight behavior, a double array of microphones has been used to accurately locate the bat (Holderied 2001). The exact position can then be calculated by triangulating, pulse by pulse, signals from the different microphones. Wide-angle microphones will enhance the chance of detecting the bat in all microphones. However, as wide-angle microphones are generally less sensitive, the range is reduced (Holderied 2001).

Broadband versus Narrowband Detectors

Broadband systems have the advantage of allowing simultaneous detection of different bats that use different frequencies. Although TE and FD systems are broadband, theoretically enabling observers to monitor the entire bandwidth used by the bats, limitations are set by the frequency response of the microphones and the filters that are incorporated into the detector to reduce noise at lower frequencies.

Tuned heterodyning systems optimally detect signals in the narrow frequency window around the tuned frequency. This is an advantage in various situations. It allows the researcher to concentrate on particular species outside the frequency range of others. Tuning to the loudest frequency can also increase detection range. The detector can be tuned outside potential sources of noise, such as traffic or insects. Conversely, bats using frequencies outside of the tuned frequency range will be missed (Fig. 7). With a handheld detector, this can be compensated for by manually scanning the frequencies of interest. For stationary detectors, a 'best setting' has to be chosen, where all or most of the expected species will be detected. In some models of heterodyne systems, an automated scanning function is available.

THE DETECTOR AS A TOOL FOR IDENTIFICATION

Many current applications of bat detectors concern linking species to their calls for species identification. These studies started with high-frequency microphones connected to instrumentation tape recorders (e.g., Ahlén 1981, 1990; Fenton and Bell 1981), and advanced to recording of frequency-division signals (Ahlén 1981, 1990; Ahlén et al. 1984; De Jong and Limpens 1985; Weid and Von Helversen 1987), to recording time-expanded signals on DAT recorders or direct recording

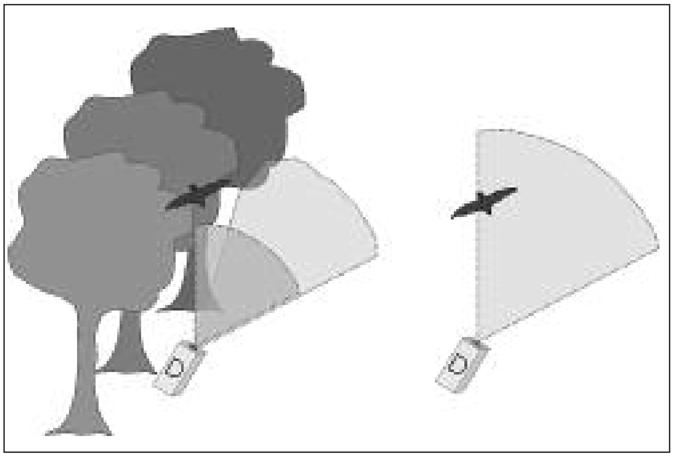


Figure 6: Nature of sampling bias as a function of habitat complexity.

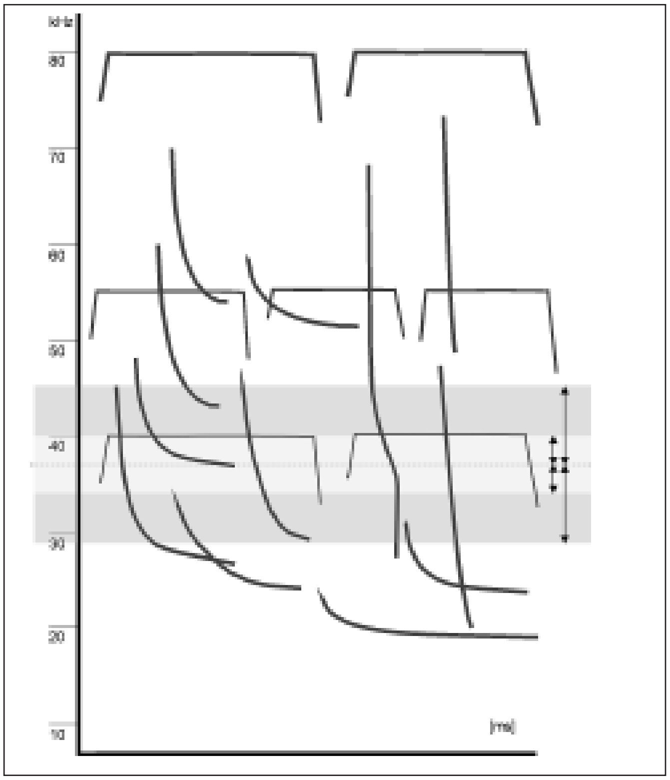


Figure 7: Schematic spectrograms of selected European species. A broadband system allows detection of signals over the entire range used by bats. In a heterodyne system, a tuneable frequency window is made audible and bats outside of this window are missed.

of ultrasound to a hard disk (Ahlén and Baagøe 1999; Limpens and Roschen 1995).

The use of a detector as a tool for species identification requires the ability to capture and process ultrasound, as well as to characterize parameters necessary for species identification. Study and description of the variation in a species' echolocation behavior require a system and microphone with the best possible capacity to capture the real physical phenomena in a single pulse as well as the pulse train. For species identification, it is often possible to work with less detailed or derived 'reproductions' of echolocation calls, as produced by FD and heterodyne detectors, as long as these 'reproductions' are sufficiently distinct from those of all other sympatric species. Parameters that allow the identification of species from their echolocation calls include the frequencies used, presence of harmonics, pulse shape, frequency with peak energy, pulse length, the length of the interpulse interval, and rhythm. For a general description of these parameters and their use for identification see Ahlén (1981, 1989, 1990, this volume), Ahlén and Baagøe (1999), Fenton and Bell (1981), Limpens (this volume), Limpens and Roschen (1995), Neuweiler and Fenton (1988), Neuweiler (1989), O'Farrell *et al.* (1999), Tupinier (1996). Here we discuss how these parameters are captured by the different detector systems for audible interpretation in the field or for subsequent analysis (Table 2).

FREQUENCY-RELATED PARAMETERS

Tonal quality[1]. To perceive the tonal quality that may be present in a signal, a heterodyne detector must be tuned to the more shallow FM or QCF part of the call. This tuning allows for a general analysis of pulse shape. The call component with tonal quality is often at the frequency with highest energy (i.e., loudest part of call). In broadband systems, the shallow parts of FM pulses or the QCF parts will have tonal quality, but the signal, as it is heard, will always be composed of all frequencies present in the output signal. This makes it difficult to focus on tonal quality in the shallow part or QCF part of a pulse in FD systems. Thus, in FD systems, fine details such as differences between similar species will be masked. In the output of a time-expansion detector, not only the frequency but also the modulation rate is lowered by e.g., a factor 10, and tonal quality will be clearly audible in all but the steepest pulses. With practice, differences in pulse shape and pitch are relatively easy to distinguish using a heterodyne or a TE detector.

Heterodyne detectors are ideal for assessing the presence or absence of tonal quality and differences in tonal quality between species of bats using FM calls. The width of the frequency window (low-pass filter) determines the performance of the detector: A relatively small-frequency window (e.g., ± 2 kHz) enhances the discriminative properties along the frequency axis, but the detector will monitor a narrower frequency band and consequently miss species at other frequencies.

The narrowband pulses in constant-frequency (CF) calls will be perceived as flat, pure tones by all 3 detector systems. Differences between stationary and flying bats, or straight and curved flight are quite obvious due to changes in frequency produced by Doppler shifts in these long pulses. Heterodyne detectors can be used to determine the CF frequency in the field, but are vulnerable to missing narrowband pulses because of their narrow listening window.

Best listening frequency[2]. The best listening frequency can be determined in the field using a tunable heterodyne detector. A relatively small frequency window enhances the discriminative properties in the frequency domain. Broadband FD or TE detectors do not provide information about best listening frequency in the field. When different species hunt together, a heterodyne detector allows the observer to focus on the different relevant frequencies of narrow frequency bands. In the slowed-down output signal of time-expansion detectors, it is possible to distinguish between the pitch of differ-

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[1] **Tonal quality**: the rate with which the frequency changes in a bat call differs between species. When this rate of change (within the converted signal) is too high for our human ears, we will hear sound phenomena without pitch and thus no 'tonal quality' is heard. When the rate is slow enough for our ears we will hear pitch and thus sound with tonal quality. For more elaborate description, see Limpens (this volume).

[2] **Best listening frequency**: a narrow frequency band within the call, where tonal quality is perceived best and where amplitude is highest, is referred to as best listening frequency. For more elaborate description, see Limpens (this volume).

ent species. But in the field, it is difficult to audibly recognize all individual species using real-time broadband output from FD detectors.

Harmonics. The ability to identify harmonics in the field enhances the likelihood of the heterodyne detector to be able to discriminate among species. When the frequencies of narrowband calls of different species are similar, the frequency 'gap' is doubled or tripled in harmonics, which can enhance discrimination. Due to the processing of the signal, harmonics are no longer present in the output of a FD system. Where multiple harmonics are present, the system divides only the strongest frequency. In time expansion, harmonics are not audible in the field, but are available for analysis in the output. With its capacity to process harmonics, this system better enables the recognition of pulses of different species overlapping in time but separated in frequency.

Frequency range. A tuned heterodyne system allows measurement of the peak frequency (F_{max}) and the minimum frequency (F_{min}) in the field, whereas TE and FD detectors do not. A computer and analysis software are needed for this to be possible. For signal analysis, one must be wary of the difficulties that a frequency-division system has in capturing rapid frequency changes, which can occur at the beginning or end of a pulse. Maximum frequency, especially, will be underestimated (Fenton 2000).

Shape or curvature. Information about pulse shape is available through analysis of TE and FD recordings, but only large differences like FM, FM-QCF-FM, FM-QCF, QCF, QCF-FM, and FM-CF-FM can be heard in time-expanded pulses. Fine details of pulse shape require post-recording analysis. The output of a frequency-division detector does not allow any measure of shape aside from the recognition of tonal quality. Working with heterodyne detectors in the field leads to an ability to interpret aspects of shape indirectly through other parameters, like tonal quality, change of frequency while tuning, or position of QCF or shallow FM in the pulse relative to steeper parts.

AMPLITUDE-RELATED PARAMETERS

Loudness, maximum sound pressure, and energy. Sensitivity and detection range differ between detector systems and brands. To interpret differences in perceived loudness, the observer must accumulate substantial experience with the particular detector (Limpens 1994, 1999; Waters and Walsh 1994).

To appreciate loudness, a heterodyne detector should be tuned to the best listening frequency, or QCF frequency, when present. Both heterodyne and frequency-division systems, being real-time systems, allow the observer to interpret what is heard while the distance to and behavior of the bat is observed directly. With a time-expansion system, observers must integrate data about the distance and behavior of the bat with the playback of the time-expansion signal at some subsequent time. In automated, stationary set ups, or in analysis of recorded sound, there is little possibility to account for how distance and behavior affect the loudness of a call.

TIME-RELATED PARAMETERS

Pulse duration, interpulse interval, and repetition rate. Both frequency-division and heterodyne systems allow observers to use interpulse interval and repetition rate features in the field. The 'slowed down' aspect of the time-expansion system enhances the possibility of measuring pulse lengths. However, due to the auditory discrimination performance of human hearing, interpretation of pulse intervals and repetition rate become difficult if not impossible because they become too long and too slow.

Recordings from time-expansion and frequency-division detectors can be used to make relatively accurate measurements of pulse duration, interpulse interval, and repetition rate. To define the beginning and end of a signal, a good signal-to-noise ratio and no overlap between signal and echo are required (Kalko and Schnitzler 1989). Because the tuning window of the heterodyne system cuts out part of the pulse, these detectors cannot accurately measure duration. However, for low-duty-cycle bats with long interpulse intervals, errors in the measurement of interpulse intervals and repetition rates will be slight.

Rhythm. The tonal quality in the real-time output of the heterodyne detector enhances the potential to interpret the rhythm of call sequences. The real-time audible output of a frequency-division detector enables an interpretation of the rhythm, but differences in tonal quality between alternating pulses are hard to perceive. As with repetition rate, interpretation of rhythm is difficult with a time-expansion system, because of the greatly slowed reproduction of the call sequences.

Whereas the human brain is good at interpreting patterns and thus an excellent tool to assess rhythm, quali-

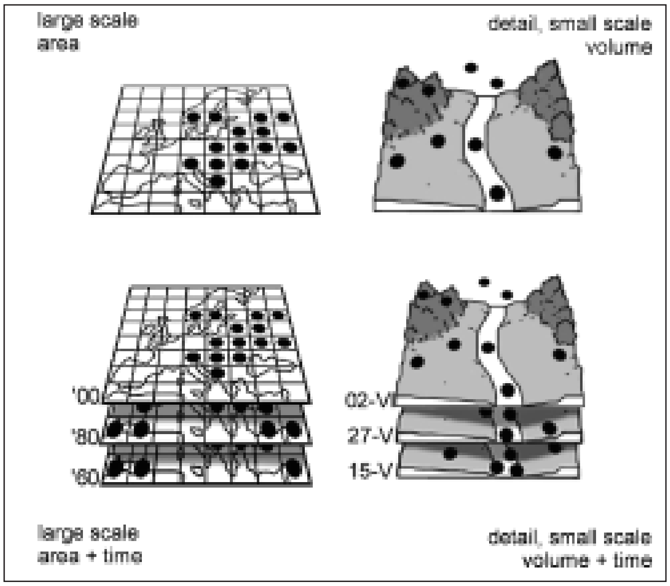

Figure 8: Scale and resolution in space and time.

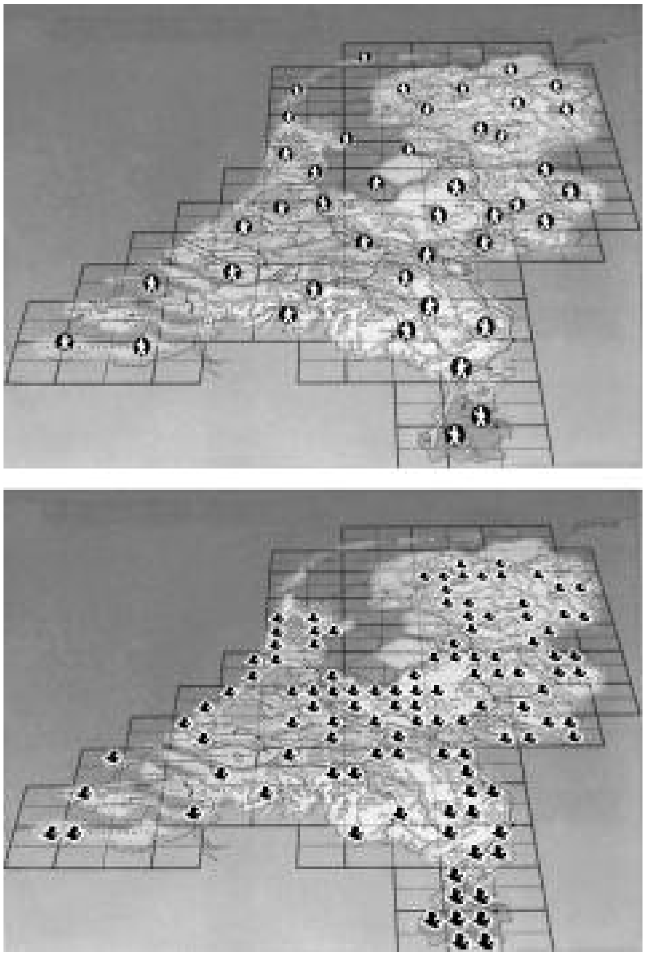

Figure 9: Assessment of occurrence and distribution of bats on a large scale.

tative assessment of rhythm is not typically used in 'manual' analysis, nor in advanced approaches like multivariate analysis or neural networks (Burnet and Masters 1999; Parsons and Jones 2000). Studies using multivariate techniques and neural networks should explore using not only single-pulse parameters, but also incorporating parameters associating pulses with previous and following pulses, and interpulse intervals.

Social Sounds

Social sounds are very species specific. However, the time-frequency structure of bat social calls is much more complex than for sonar signals, and therefore not always easy to interpret. Time expansion allows for measuring the time-frequency structure in the field, while heterodyne detectors provide the possibility to focus on specific frequency bands to enhance discrimination.

CHOICE OF DETECTORS

Working with bat detectors involves more than just choosing a heterodyne, frequency-division, or time-expansion detector that will convert the ultrasonic signal to within our range of hearing. In addition to the detector system, issues that must be addressed include the deployment of the detector(s) and the experimental design that will best answer the research question(s).

Different microphones range from relatively cheap electret microphones to expensive and sophisticated custom-made capacitance microphones. Recording devices also vary with the options, including cumbersome and expensive analogue instrumentation tape recorders, professional tape or cassette recorders, DAT or minidisk recorders, or direct recording to hard disk. Software is also needed to visualize and analyze the recordings.

The choice of a detector or combination of detectors must be made in response to the constraints of budget, deployment, mobility in the field, capability of following bats versus recording at one location, the setup of transects or sample points, the number of observers, and the level of training of observers. Also, will species identification be based solely on the observer's interpretation of sound and flight behavior or on automated analysis with a computer? Will the data be analyzed in the field or in the lab? The answers to these questions point in different directions, and no simple answer or best solution always results.

RESEARCH QUESTIONS

Information about bats in their landscape differs in scale and detail. Therefore, the questions we ask must address different levels of scale and detail. Questions may concern the spatial use of habitat, with the scale ranging from individual's use of feeding habitat to the geographic distribution of the species. Issues expand from static to dynamic with questions such as "are distributions and population sizes changing" or "how do populations functioning ecologically and dynamically." From distribution to trend, from occurrence to habitat use, and from ecology to population dynamics, there are transitions in scale and detail.

We have attempted to summarize a range of possible research questions at different levels of resolution in space and time and to identify the most appropriate and inappropriate detector systems for these applications

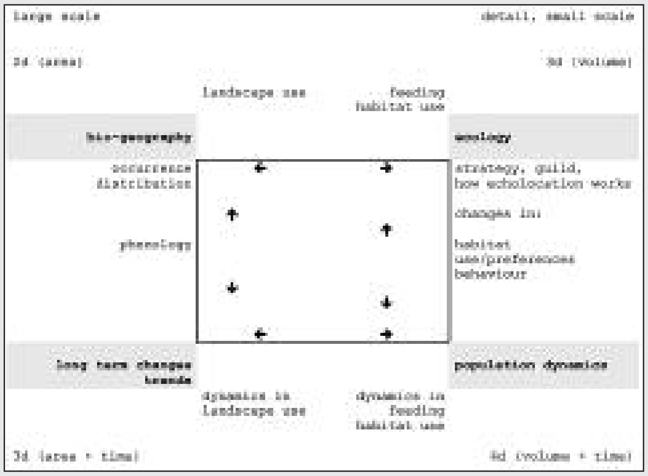

Table 3: Possible research questions with regard to scale and resolution in space and time.

(Table 3, Fig 8). This summary can serve as a tool for selecting appropriate detectors. As examples at different ends of the spectrum of typical applications, questions that concern biogeography or the presence of a species in different habitats (upper left corner, Table 3, Fig. 8), require discriminative parameters of calls, but it is not necessary to identify all calls or to necessarily have the best characterization possible of call features. If large areas are to be surveyed, high mobility and several observers are needed, suggesting the need for multiple detectors of relatively low cost. In this situation, a successful approach could involve heterodyne detectors supported by some sets of TE or FD detectors for recording and back-up analysis (Fig. 9; Limpens et al. 1997). In contrast, questions about ecology and differences in echolocation calls within the context of how species fit into feeding guilds (upper right corner, Table 3, Fig. 8) demand high-quality recordings with the best acoustic information possible. Between the 'extremes' in the upper left and upper right corners of Table 3, different research approaches are possible. These approaches include surveys or transects with heterodyne detectors and combinations of heterodyne with FD or TE for back-up analysis (Limpens 1993; Walsh and Catto 1999; Walsh and Harris 1996a, 1996b), to setups with arrays of stationary systems involving TE, FD, or heterodyne detectors generating automated recordings on DAT or computer (Britzke et al. 1999; Jones et al. 2000).

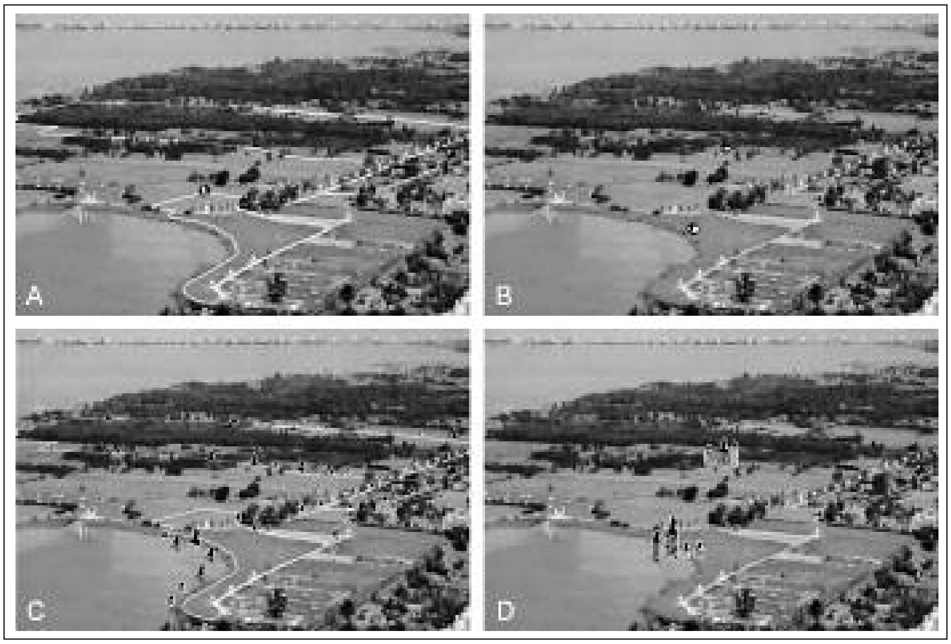

Figure 10: A mobile survey with a focus on what is perceived as 'good' bat habitat will lead to an assessment which is different from stationary set ups. The mobile approach will lead to a good cover of the bat-occurrence landscape use in an area (**A**, **C**), whereas the stationary set up can provide detailed, quantitative data regarding the sample point (**B**, **D**). The observed spectrum of species might differ, where the mobile approach provides the opportunity to investigate particular habitats for target species.

Where habitat use is the focus, a number of stationary detectors (i.e., sample points) may be the most appropriate. It is worth noting that an expensive detector at a fixed position will not monitor all habitats around the sample position equivalently, and sampling bias could lead to a different spectrum of species observed. Nor will the detector document differences in echolocation behavior in relation to flight patterns within various habitat features around the sampling point. In such cases, the use of stationary detectors on fixed sample points coupled with observers with the capability of TE recordings, would be complementary.

Recent work demonstrates that studies of habitat use must take into consideration that bats use different strata, ranging from near the ground, to thousands of meters above the ground (Fenton and Griffin 1997; Griffin and Thompson 1982; McCracken 1996; Menzel et al. 2000). Because of the remote nature of altitudinal surveys, visual observation to enhance identification and tuning of the heterodyne system are difficult or impossible. Therefore, the use of broadband detectors is advisable. Although the detection range is limited, frequency-division systems seem most applicable to remote monitoring of bat activity because they are continuously monitoring the landscape. Where species identification is required, time-expansion systems may be preferred because they provide maximum information. In a time-expansion/heterodyne combination, the latter system continuously monitors the landscape, thus compensating for the time-sampling and non-real-time performance required of the time-expansion system.

In situations where bat activity is within, above, or near structures, such as roads, tunnels, wind power utility structures, caves, etc., relatively simple stationary automated setups with heterodyne detectors and signal-activated recorders can be effective, with the use of time expansion for back-up recording of high quality signals (e.g. Bach and Burkhardt 2000; Bach et al. 1999, 2001; Rahmel et al. 1999).

Long-term observations are needed to document changes in activity patterns and trends over the course of seasons or years (Table 3, Fig. 8). Such studies might concentrate on where stationary setups are feasible. Where activity patterns and trends in populations in larger areas are involved, larger numbers of sample points, transects, or observers are needed. These characteristics require low profile and low cost approaches, where an accurate assessment of relative bat density rather than high-tech processing of sound is needed. Here again, heterodyne detectors, supported by some sets of time-expansion (or frequency-division) detectors for recording and back-up analysis can be a successful approach (Walsh and Harris 1996a, 1996b; Walsh and Catto 1999; de Wijs 1999).

When the dynamics of habitat use are of interest (bottom right corner, Table 3, Fig. 8), the data should provide high resolution in time and space, but possibly at a smaller scale. In such situations, automated set ups with a relatively high number of sampling points will ensure the acquisition of high-resolution data. At the same time it may also be necessary to identify species in cluttered habitats where they are least easy to identify. The stationary setups should therefore be based on time-expansion or frequency-division systems to allow for signal analysis.

In the transition towards the bottom right corner (Table 3, Fig. 8), where the dynamics in landscape use are of interest, we are dealing with a larger scale and less resolution in time. Rather than more sophisticated processing of ultrasound, key factors involve the number of sample points in a landscape and the intensity and cost effectiveness with which samples can be collected, either automatically or manually. In manual surveys involving transects or sample points, a successful approach can involve multiple observers with heterodyne detectors. Where automated sampling is employed, relatively simple, stationary, automated setups with heterodyne detectors and signal-activated recorders can be effective. Both manual and automated surveys should be supported by some sets of time-expansion or frequency-division recordings for back-up analysis and verification of the composition of the bat fauna.

ACKNOWLEDGEMENTS

We thank the organizers of this symposium for creating a great forum for discussion and exchange. Lothar Bach, Brock Fenton, Marc Holderied, Lars Pettersson, and Peter Twisk shared information and commented on earlier versions of this manuscript. Peter Twisk provided beautiful illustrations. The manuscript benefited from the comments and careful editing of Mark Brigham and two anonymous reviewers.

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ARE ACOUSTIC DETECTORS A 'SILVER BULLET' FOR ASSESSING HABITAT USE BY BATS?

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The current state of our knowledge regarding bat ecology is based largely on advances in research techniques that include mist netting, radiotelemetry, and recent refinements in acoustic-detector technologies. The recording and analysis of ultrasonic calls has become a commonly used tool for conducting simple surveys as well as investigating the ecology of bats. In particular, these tools are being used increasingly to address differential use of habitat by bats and to infer patterns of bat activity and behavior. However, recent criticism of the application of acoustic tools implies that their use for addressing differential use of habitat may be limited. We assessed 46 papers published between 1980 and 2001 that make use of acoustics to address questions regarding use of habitat by bats. We summarize methods applied by these authors and assess their utility to address stated research objectives. Many papers attempted to apply survey or activity methods to questions that required a more complex study design. In comparing methods from these papers in the context of design criteria, many authors failed to satisfy some if not all of these criteria. One of the most common omissions was a clear articulation of the limitations of the acoustic equipment employed. Symposium participants provided additional assumptions that could be acknowledged during the creation of a study design. Failure to acknowledge and understand the limitations of acoustic tools can lead to erroneous conclusions and ultimately to inappropriate management.

Key words: acoustics, Chiroptera, echolocation, habitat use, North American vespertilionids, technology, ultrasonic detector
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INTRODUCTION

When investigating questions about ecological patterns or processes, investigators often look for a means to view the complex world simply. Although the world of bats may be more complex than other systems because bats operate largely in 3-dimensional space, the basic rules of science still apply. The science must be able to be replicated, and the methods and equipment used must be reliable.

Over the last few decades, a number of acoustic technological advances have occurred. How has this technology been applied to the ecology of bats? Several publications have recently appeared (e.g., Barclay and Brigham 1996; Gannon and Bogdanowicz 2000; Jones et al. 2000, Jones et al, this volume, Thomas et al. 2004) that highlight use of acoustics to address questions regarding the biology of bats. Scores of people have learned to operate various types of detectors and published results of their efforts in the scientific literature. Since 1980, at least 46 papers have appeared in which acoustic detectors are the dominant tools used to assess habitat use by bats. It appears that some researchers and resource managers consider acoustic detectors a "silver bullet" for understanding bat movements and habitat requirements. A perception is that acoustic detectors provide large quantities of data about the ecology and behavior of individual bats (Corben and Fellers 2001, Fenton 2000). However, sometimes the use of acoustic detectors is misdirected.

Gannon et al (2003) summarized assumptions that have been formulated for survey design and applied them to an assessment of published papers whose objective was to assess habitat use by bats using acoustic detectors. Gannon et al. also produced numerous models and discussed the effect of model construction and generation of false models based on altering assumptions (Gannon et al. 2003; Sherwin et al. 2000). In this review, we report the results of a formal examination of published papers about studies which employ acoustic detectors to evaluate habitat use by bats. We also summarize the comments of participants in the Echolocation Symposium directed at applying acoustic detectors in ecological studies.

MATERIALS AND METHODS

We reviewed 46 published papers from common, peer-reviewed scientific journals and published symposia that used acoustic techniques to investigate habitat associations of bats (Table 1). We evaluated each study for common strengths and faults, study design and statistical analyses, in the context of a list of study design assumptions (Figure 1, Table 2). For each paper, information on location of study, species considered, dates during which the study was conducted, year the study was published, stated hypotheses, and scale of inference was recorded. Furthermore, we recorded if the unit of "call" was described or which call type examined was stated, how captures were defined and treated statistically, how calls were identified, how habitat volume was surveyed, and whether the data were considered continuous or categorical. The amount of replication was considered, as was differential probability of detection of acoustic signals by grouping (that is, by species, species group, sound group, or guild). In addition, temporal scales were addressed by reviewing sampling effort (number of years a site was sampled, number of samples within a year, number of nights within a sample period, and duration of sampling each night). Spatial scales were evaluated by recording how many habitat types were evaluated and how many sites per habitat were sampled. We also noted if acoustic detectors were the only tools, the primary tools, or if they were used with a variety of sampling methods, and level of inference for which results were applied. Study design was designated "inappropriate" if more that 50% of these criteria were not met. Studies where more than 50% of the criteria were stated and could be categorized were classified as "clear" and "repeatable;" those that did not were "muddled" and "unclear."

Comments at the Echolocation Symposium were solicited from attendees during several periods of discussion asking for suggestions and examples of assumptions that they make when conducting studies using acoustic detectors to evaluate habitat use by bats. In addition, participants were urged to submit comments in writing during the meeting and by email.

Journal	N	Percentage of total (%)
Acta Theriologica	1	2
Bats and Forests Symposium I	9	20
Behavioral Ecology and Sociobiology	2	4
Biological Conservation	2	4
The Canadian Field-Naturalist	4	9
Canadian Journal of Zoology	5	11
Conservation Biology	1	2
Ecology	2	4
EcoScience	1	2
The Journal of Applied Ecology	1	2
Journal of Mammalogy	4	9
Journal of Zoology	3	7
The Journal of Wildlife Management	6	13
Mammalian Biology	1	2
Nature	1	2
New Zealand Journal of Zoology	1	2
Plant Ecology	1	2
Wildlife Society Bulletin	1	2

Table 1: List of 18 journals from which 46 papers where selected to examine the use of acoustic detectors to evaluate habitat use by bats.

RESULTS

The papers we reviewed were parsed into 3 categories: 1) studies investigating specific call parameters to infer species response to various habitats (n = 4); 2) studies focusing on habitat associations of a single or paired species (n = 10); and 3) community level studies investigating distribution and relative abundance of multiple species across the landscape (n = 32). Category 1 papers satisfied all criteria and study-design assumptions. In general, these papers were easy to follow, had clearly stated goals, and explained how those goals were met. Papers in category 2 were mixed in that half defined assumptions (even partially), call units, and management recommendations. Approximately ⅓ of these papers defined the level of biological significance that calls represented (calls represent some unit such as a species, guild, etc), however, regardless of earlier statements, in all papers in this category, authors concluded by identifying calls to species. It was assumed in all papers that bats were randomly distributed in 3-dimensional space and that individuals were equally detectable at all heights. In Category 3, 3 of 32 papers (8%) defined the biological unit that a call represented, addressed the question of 3-dimensional space, or addressed concerns regarding temporal variation across multiple years. Less than half (42%) of these papers defined a call, assigned specific designations to calls, or had sufficient replication to test stated objectives. Many papers inconsistently used a combination of specific and group assignments to calls; however, most were muddled and did not provide enough detail about study design assumptions for a complete evaluation. The majority of studies (>75%) failed to acknowledge limitations of the acoustical data owing to conclusions about larger scale applications than the sampling design and subsequent analyses could support. Often, studies included recommendations for landscape-level management despite data collection scaled to point (local) sampling. These papers typically used acoustic tools exclusively. Most importantly, none of these papers listed assumptions made during data collection and/or analyses, even though 13 of 32 acknowledged limitations of acoustic tools. The most common difficulties in category 3 papers included: lack of replication, pseudoreplication, lack of clear hypothesis testing, and nonstandardized techniques for data collection. As a result, we conclude that 20 of 32 (63%) of these studies were designed inappropriately and 29 of 32 (89%) of these papers make unsubstantiated generalizations about differential use of habitat by bats.

DISCUSSION

Failure to address variation in bat activity across spatial and temporal scales was nearly universal in papers published prior to 1999. The most severe problems in our view are a failure to address the assumptions necessary for statistical analyses or study design requirements for replication. Assumptions about the limitations of acoustical equipment and how it is implemented relative to the question being addressed were rarely linked.

Studies that investigate specific aspects of echolocation and impacts of those findings on habitat use (Category 1) and papers that focused on 1 or 2 species (Category 2) withstood scrutiny far better than those whose objective was to investigate community-level patterns of habitat use by bats at noncommunity-level scales (Category 3). Category 1 and 2 papers were more detailed regarding study design and had clearly defined, testable hypotheses. Another interesting pattern concerned the quality of "early" studies (1980 - 1992) compared to those conducted more recently. Early studies tended to focus on testing plainly stated hypotheses. Early studies that involved basic inventory of bat communities did not rely on acoustic tools exclusively. Most early acoustic work employed bat detectors as secondary or even tertiary devices. Our interpretation of this is that the cost of early bat detectors and associated "user-unfriendly" hardware made this equipment novel, but of secondary importance. Acoustic detectors may have been considered an interesting side project, but one that still needed serious attention to study design. Most of the papers in Category 3 lacked a clearly stated hypothesis and/or a description of sampling design that could be followed by the reader. This made the task of determining how the study was actually conducted quite challenging.

The proliferation in apparent misuse of acoustic tools may simply reflect an increased use of detectors as cost has decreased and easier user interfaces become available. It is relatively simple using current applications to collect large amounts of data (particularly when compared with mist netting and radiotelemetry). When new techniques or new applications of old techniques become available, initial misuse may be observed. For instance, metal bands were used for years (1930s to 1970s) to individually mark large series of individual bats. However, some bands irritated some individuals to the point of injury, which affects behavior and perhaps survey outcomes by causing additional mortality. Alternatives such as plastic bands, lipped metal bands, and necklaces have replaced straight-edged metal bands in many cases (Barclay and Bell 1988). Was the arrival and application of a new device (metal bands to the wings of bats) a case of a technological misuse? In some cases, yes; but in retrospect, better techniques and technology (plastic bands) improved banding methods and results.

Hayes (1997) was the first to publish guidelines about temporal variation for monitoring levels of bat activity using acoustic detectors. He emphasized the need for repeated monitoring of sampling locations using acoustic detectors. Failing to do so limits the infer-

Paper # _______ Authors__________________________
Year_________

Title____
General location where study occurred
____
Date of Research ____ Study Duration(yrs, wks, hrs) ___
Stated Hypothesis/Question________________________
Scale of Inference (local? landscape?) _______________
Number of species involved _______________________
1. Unit of call described by authors? ________________
2. Are captures treated as discrete/independent? If no, how described? _________________________________
3. How is a capture defined (call, pass, sequence)?
____
4. Call type measured (search, pursuit, terminal, ALL)
____
5. How calls are identified (species, guild, other)
____
6. Was habitat defined in 3D, then surveyed in 2D?
____
7. Are data considered continuous or categorical?
____
8. Was there replication/repeated sampling of a site or habitat?_____________________________________
9. How are species treated regarding probability of detection? _______________________________________
10 Spatial and Temporal aspects:
- How many years did the investigators sample the study site ____
- How many times within a year was the site(s) sampled ____
- Number of nights within sample time the authors ran detectors ______________________________________
- How many hours each night did the authors run the detectors ______________________________________
- Number sites, habitats, elevations, authors sampled ____
- Number times within a year the site (as above) was sampled _____________________________________
- Number of nights within habitat, elevation detectors run ____
11. Inference – Was it a point study? Landscape study?
____
12. Did the authors stay within the bounds of their study? ____
13. Were management recommendations made?
____
14. Were acoustic tools primary or accessory to study?
____

Figure 1: The standard questions asked of all reviewed papers. Summaries are provided in text.

ential power of data. Hayes (1997) noted that because of the range of inherent variability in data collected at a single point, this type of sampling may obscure patterns or lead to inferences about variation of biological significance incorrectly. Without an estimate of within habitat variation (by comparing mean levels – and variance – of use of homogenous units), between-habitat comparisons cannot be made. Randomization cannot replace the use of multiple units in a habitat, because no estimate of within-habitat variability can be made using just 1 or 2 detectors. Typically, in category 2 and 3 papers, single detector systems were used to collect data and then findings were compared across time and space to assess differential habitat use. Failure to account for temporal variation at sampling locations presumes that use of habitat is static. Gannon et al. (2003) argue that to assume static spatial and temporal use of habitat by bats is inaccurate. Moreover, failure to account for spatial and temporal variation results in generation of false models of habitat use (at all levels of call classification).

ARE ACOUSTIC DETECTORS THE SILVER BULLET?

Due to observed spatial and temporal variation in bat activity (both within and across habitats), concurrent sampling should be adopted whenever possible. Sampling points need to be established *a priori* with sampling effort equally distributed among points. Concurrent data collection at multiple sampling points is the best form of replication. Without an estimate of within-habitat variation, between-habitat comparisons cannot be made. In cases where concurrent data collection is not possible (for example, because of equipment costs, limited access to sites, or endangered species), alternative techniques may be available (some of these issues are addressed by Jones et al. this volume). In most cases, the study should not be done if the study design is inadequate to address the question or if the equipment inventory is insufficient. Clearly, researchers using acoustic detectors to draw conclusions about habitat use by bats must address the underlying assumptions made when conducting their studies. Clearly stating these assumptions not only assists other scientists to evaluate how the study was conducted, but serves to anchor conclusions in biological reality. This helps to ensure that the data will be applied in context. Sherwin et al. (2000) emphasized that data from acoustic studies may generate patterns, which are artificial. Furthermore, the assumptions may preclude the exclusive use of acoustic detectors for investigating differential use of habitat by bats. However, even limiting or unrealistic assumptions should be listed if they are relevant to the study design (Hayes 2000).

We provide an example of how assumptions (Table 2) can be defined for use in echolocation-monitoring studies that use acoustic detectors. Assuming captures of call sequences are correlated with habitat type (Assumption 1) allows the investigator to align the proper classification (such as guild or ensemble) for calls. This assumption is valid unless habitat is a narrow strip of vegetation (such as a wind row or strip of trees bordering successive clear cuts) where habitat may appear distinct, but from the scale that bats operate in, may in fact be discontinuous (Assumption 6). We considered each call sequence to be independent of the next (Assumption 2). This is a valid assumption as long as bat activity is not high. With high bat activity, many con-specifics as well as multiple species fly over detectors simultaneously. High calling activity is when more than 2 bats (or 2 species of bat) are calling in a call sequence at 1 time and these multiple bats will be represented on a single call sequence file. If multiple detectors are operating concurrently, examination of call sequences containing search phase call-types (Assumption 4) will provide additional information to determine if calls are independent. Moreover, if sets of detectors are run for multiple nights concurrently at different sites within the same habitat type, true replication has occurred (Assumption 8). If this same set of detectors are maintained and operated over time to include multiple seasons and multiple years, then spatial and temporal scales can be examined for call-sequence data (Assumption 10). If sequences of multiple bats cannot be determined to be independent (contradicting Assumption 2), calls and call sequences should be discarded from further analysis. Captures can be defined as a discrete sequence of search-phase calls (Assumptions 3, 4, 7).

How call sequences are defined (we observed 5 groups in the literature: species, species-groups, sound-groups, guilds, and all calls; Assumptions 5 and 6) can result in dramatically different conclusions. If investigators cite the convention that they follow, then their study can be interpreted at the proper scale. Calls are the unit of classification, not species or bats. For instance, identifying call sequences to species has the most biological significance at the local level. For investigations at the landscape level, guilds (or bat ensembles; Patterson et al 2003) allow ecological inference at a coarser scale. Early papers using heterodyne detectors may have actually followed a guild concept (in theory) by identifying calls as sound groups (popular categories were 25 and 40 kHz). This manner of grouping calls provides a repeatable method of classifying acoustic data but offers little resolution on the question of differential use of habitat by species of bats.

Other considerations when using detectors include differential detection of lower intensity species (e.g., *Corynorhinus*) or bats that are specialized aerial hawkers (e.g., *Tadarida*; Assumption 9). Not all species are detected equally by acoustic detectors and this is a bias that must be adressed. The study design and the resulting interpretation of call data should provide compensation for differentially detectable species.

We stress that any study that produces extensions of species ranges, suggests management needs, or even documents new aspects of natural history, should not be based on acoustic data alone (Assumption 11). Acoustic detectors are important for inventory, locating sites for further study, or for identifying concentrations of activity for bats in general (also for species or guilds of interest in particular). However, addressing more complicated ecological questions involving bats requires additional techniques. Other devices and techniques should be used to assess habitat use (e.g., using radiotelemetry, infrared photography, or satellite tracking), while recognizing that these methods have biases as well.

During the Echolocation Symposium, a number of attendees suggested additional assumptions that should be considered when contemplating using acoustic detectors to study bat ecology. Although a few novel assumptions did arise, most of these suggestions were specific cases addressed by the more general assumptions that we have presented (Table 2). We describe those additional considerations and the others in the context of the assumptions.

Several participants raised the concern about evenness of detection of the acoustic signal by the detector. Assumption 9 states that all calls have the same probability of detection. These concerns were: A) Clutter assumption: The call remains constant regardless of background clutter (acoustically, clutter consists of other echoes sensed than those from the target). If the assumption is made that the bat does not adjust intensity (hence detectability) based on the amount of clutter, then all calls are equally detected from that bat. B) Directionality assumption: There is no variation in the directed emission of the call from the bat during flight. This assumes that the bat emitting the call is always oriented towards the microphone. C) Sound assumption: Sound spreads evenly from the time it is emitted by the bat. This assumes that all sound signals are acted upon in the same way by temperature, wind, moisture, vegetation, and other factors, so that signals are all received in the same way by the acoustic detector. Sounds that are detected are actually sounds being produced and used by the bat. D) Community assumption: When recording at the level of a bat community, no other influences determine characters of calls of bats other than the evolutionary influences of 1 species of bat on another.

Assumption 5 (Table 2) states that the study will designate the level and method used to identify calls that are being detected. A corollary to this was suggested; E) Variation assumption: There is no geographic variation within a species to confound identification based on acoustic characters. Taxonomic constraints limit variation within a species; however, local evolutionary pressures act to shape the call in the community where it has evolved.

	Assumption
1	How will a capture be interpreted?
2	Will captures be treated as independent events?
3	How will a capture be defined (minimum number of calls, a pass, etc.)?
4	What call sequence type will be measured or assessed (i.e., search, pursuit)?
5	How will ‘difficulties’ of call sequence identification be resolved (i.e., could be addressed by assigning calls to classification groups or guilds)?
6	How are different types of habitat defined (2- or 3-dimensional spaces)?
7	Will data be considered categorical or continuous?
8	How will replication be achieved?
9	Will all species (or other category) have the same capture ability or probability of detection?
10	How will spatial and temporal variability be addressed (Hayes 1997)?
11	What is the desired scale of inference?

Table 2: The strength of inference resulting from echolocation-monitoring studies is minimally a function of the extent to which underlying assumptions are met. Here is a list of 11 assumptions that we used to evaluate published literature on the use of acoustic detectors in studies of habitat use by bats.

In addressing temporal and spatial variability, we suggest that multiple detectors be run concurrently within each habitat type over time. To this end: F) Randomization assumption: Randomization of multiple detectors through space is necessary.

Several novel assumptions were voiced regarding the recording equipment itself and non-data. G) Equipment Assumption: All detectors (of the same design type) detect calls equally. This assumption demands that detectors are routinely maintained and calibrated. Equipment throughout the recording chain does not affect character of the calls. H) Ghost bat assumption: The individuals that represent the species of interest are present in the sound space of the study site. Furthermore, it is assumed that if a particular species is not detected during the acoustic-survey period, that does not mean that species is absent from the community.

Lively discussion has ensued, both in the literature and at the meeting, about the level at which calls can be identified. Identifying bat calls to species can be challenging for those who are not experienced or are not trained in systematics. For these reasons at least, providing calls identified to species from calls recorded by acoustic detectors to management agencies may seem to pose too great a risk and should be considered carefully.

For instance, we recorded a single *M. sodalis* at a study site that included few call-sequence files, but we were not comfortable using these reference call sequences recorded from this individual to categorize *M. sodalis* at other sampling locations (Foster and Kurta 1999; W. L. Gannon, in litt). Because of the conservation implications of falsely identifying this or other listed species by acoustic means only, it is best to be conservative. We recommend recording additional calls from other individuals and that refining characters of identification for this species should be a high priority for future surveys.

One of the unifying principles from this summary of published papers and the Echolocation Symposium is that good science requires that authors document their work through the use of a study design that explicitly states assumptions under which they are operating and archive call-sequence files generated during the study. Based on the methods and categories that we used in our examination of literature, the publications listed in Appendix 1 can be evaluated by the reader to determine if we have provided an accurate assessment of the use of detectors in ecological studies. A number of papers that we assessed came from the *Bats in Forest Symposium* (20%, Table 1; Barclay and Brigham 1996). Although papers in this volume were not peer reviewed, they are often cited in peer-reviewed literature and referred to by management professionals. In our view, there are papers in that publication that provide examples of the full range of attributes that we discuss here; it provides examples of papers with excellent study design, as well as ones that need improvement.

What becomes of data generated during surveys and other field applications of acoustic detectors? Investigators should voucher call files allowing data to be available for others to use. While some studies that we examined (5) did archive calls, the majority of papers did not describe the fate of the media that they used for data collection. As with natural history collections, a call sequence that is organized and housed accessibly allows data to be re-analyzed or mixed with data from other studies. Less obviously, vouchering calls into a call library or systematically organized informational retrieval system provides a large database that may allow comparisons across temporal and spatial scales that have never been thought possible before (see Waters and Gannon this volume)

Acoustic detectors *are* the Silver Bullet when it comes to detecting presence of bats in the field quickly. Given careful attention to study design and inherent assumptions, detectors can be used to collect data about patterns of use of habitat and species movement through time. However, there are pitfalls and hazards in trying to address questions about bat ecology, especially aspects of assessing habitat use. That road however, can be made much smoother with a readable road map *vis a vis* the statement of clearly identified assumptions applied to the study design scaled to the hypothesis or question being addressed.

ACKNOWLEDGMENTS

We are grateful to B. Keeley, Bat Conservation International, and the National Fish and Wildlife Foundation for their efforts to organize the symposium. The National Aeronautics and Space Administration (NASA), PURSUE program, and N. Nadiee, University of New Mexico (award number PP-114-01SU) provided financial support for E. S. Greenlee, who tirelessly sorted incoming call-sequence files and cleaned headers. Thanks to L. T. Arciniega, N. M. Gannon, B. T. Gannon, and L. T. Gannon for providing the basic assumptions of life (from WLG). We especially acknowledge the hard work put forward by those authors whose papers we reviewed, all of which have furthered our understanding of the potential of acoustic tools.

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FIELD IDENTIFICATION: USING BAT DETECTORS TO IDENTIFY SPECIES

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Bat detectors allow aural observation and enhance visual observation of bats in flight. The adaptation of different species to specific prey or habitat and the functional relation between their echolocation and flight behavior to these situations is recognized as the basis for identification. At the same time, behavioral flexibility and convergence of species provide a basis for confusion. Using both aural and visual cues in a comprehensive approach, identification of flying bats in their natural environment is possible. Analysis and quantification of recorded calls add to the possibilities for identification, but are of limited use without the required field observations. Repeated study of the behavior of known bats in different flight and hunting situations is needed for both the description of a bat's "vocabulary" and the observer's learning process. Knowledge about how echolocation works is needed to interpret behavioral adaptations to the habitat structure. Based on the sample of possible cues available from bats of known identity, a working hypothesis may be developed to identify bats in different situations. Feedback through other methods of identification, such as netting or tracking to roosts, is used to enhance the number of situations where identification is possible and to increase confidence in identification. Basic features or parameters of use in identification include: the bat's flight image and appearance (the size, shape, and color of the body and wings), flight style (the species' flight and hunting behavior), echolocation behavior, and social vocalizations. Information on individual species is provided to illustrate the protocol. Examples demonstrate the comprehensive approach to identification in the field.

Key words: bat detectors, Chiroptera, echolocation, flight, identification, pulse design, specific aural and visual parameters
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INTRODUCTION

Bat detectors are tools that enable us to study bats in their natural dark environment. Bat workers have progressed from using detectors to measure activity of readily distinguishable groups or to follow flight paths from known roosts into the field (Voûte 1972; Kunz and Brock 1975) to using them for species identification. This is a valuable development, because the possibility of observing and identifying bats in flight provides additional approaches to study their occurrence, distribution, ecological requirements, and conservation (e.g., Kapteyn 1995; Limpens et al. 1997; Verboom 1998; Walsh et al. 1995). Bat detectors also offer ways to engage professionals and amateurs alike in enjoying and studying bats.

Since the early pioneers in identification of bats by their echolocation calls (Ahlén 1980a, 1981; Fenton and Bell 1981; Hooper 1969, 1981), we have learned much about the complex functional and ecological flexibility of echolocation systems (e.g., Neuweiler 1984, 1989; Schnitzler 1986; Simmons and Grinnell 1988; Simmons and Stein 1980). While research into the potential and limits of identification of bats has evolved (e.g., Ahlén 1990, 1993; Ahlén and Baagøe 1999; Barataud 1992, 1996; Limpens and Roschen 1995; Limpens et al. 1997; O'Farrell et al. 1999), data about variation in calls and echolocation behavior have fueled criticism and debate about the practicality of identifying bats using echolocation calls (e.g., Barclay 1999; Vaughan et al. 1997). Thus, verification of identification is a crucial topic.

Observation and ultimate identification of echolocating bats involve a number of stages. These include: (1) interpretation of what is seen and heard by an observer in the field; (2) complementing such observations with analysis of recorded sound, either in the field or sound laboratory (Ahlén 1980a, 1981, 1990; Ahlén and Baagøe 1999; Limpens and Roschen 1995); (3) identification of recorded sound through direct analysis and interpretation on a computer screen in the field (e.g., Britzke et al. 1999; O'Farrell et al. 1999); and (4) analysis of recorded sound through multivariate or discriminant function analysis (Jones et al. 2000; Parsons and Jones 2000; Vaughan et al. 1997; Zingg 1990) or neural networks approaches (Burnett and Masters 1999; Parsons and Jones 2000).

In this paper, I focus on identification of bats in the field based on observation and analysis of sounds and flight behavior (stages 1 and 2 above). I describe an approach for identification and provide information on species for illustrative purposes. As a consequence of the geographical region in which I work, my experience and examples are predominantly European. I expect that the principles described regarding identification will hold true for any regional bat community, but that limitations will clearly vary according to the species richness of the community. This paper presents a background to the comprehensive approach to identification of bats in flight. Ahlén (this volume) provides more detail and examples regarding different European species. Limpens and McCracken (this volume) discuss the performance of different detector systems for capturing and revealing the sound parameters used in identification.

SPECIES IDENTIFICATION

Bat detectors allow us to hear and record echolocation calls and to combine these observations with information about the bat's appearance and flight behavior. A handheld detector, with at least some directionality for the microphone, helps locate and visually observe the bat in its habitat. In some situations, flying bats also produce social sounds, which can often be heard with the unaided ear. The combination of display flights and sounds are often useful in identifying bats in the field.

A Basis for Identification

The morphology, flight, and hunting behaviors of bats are linked to their echolocation behavior, a package that influences patterns of habitat use. To get the "right answer" (information contained in an echo) in a given acoustical environment (habitat structure), or in a certain phase of hunting prey (detection, tracking, interception), bats must use the "correct" signals. To hunt efficiently in a given habitat, the bat has to fly in a particular way and must have the corresponding wing morphology to do so (e.g., Aldridge and Rautenbach 1987; Baagøe 1987; Fenton 1986, 1988; Griffin 1958; Habersetzer 1986; Holderied 2001; Neuweiler 1983, 1984, 1989, 1990; Neuweiler and Fenton 1988; Norberg 1987, 1998; Norberg and Rayner 1987; Rayner 1987, 1991; Schnitzler 1986; Schnitzler and Flieger 1983; Simmons and Grinnell 1988; Simmons and Stein 1980). Echolocation and flight-behavior characteristics can therefore provide some indication about where (in what habitats and in which situations) bats may be observed. Although bats certainly are, and need to be, flexible, in most cases, a species hunts in habitats to which it is best adapted, using the optimal echolocation calls for the specific acoustic environment. This situation is the basis for identifying bats in flight.

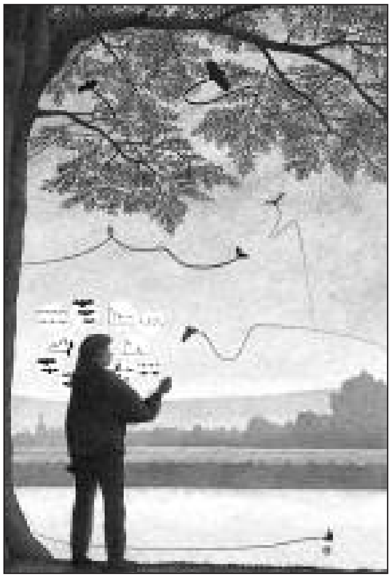

Figure 1: To identify flying bats in the field, a combination of aural and visual cues about echolocation calls and flight behavior are used.

A Basis for Confusion

The identification of flying bats requires some basic knowledge about their morphology and echolocation behavior, as well as an understanding of the theoretical background of echolocation and flight. The relation between signal design and habitat produces variation within any species, and some convergence in signal design when different species hunt in similar habitats (Ahlén 1980b; Fenton 1986, 1988; Kalko and Schnitzler 1993; Kapteyn 1993; Neuweiler 1983, 1984, 1989, 1990; Neuweiler and Fenton 1988; Obrist 1995; Rydell 1990; Zingg 1990). For field identification, as well as sound analysis, we must relate behavioral adaptations of sound and flight to habitat in order to ensure correct interpretation and use of data on echolocation, flight, and hunting.

A COMPREHENSIVE APPROACH

Field identification of flying bats should be based on all available cues, including echolocation sounds, appearance, and flight behavior. I prefer to use a heterodyne and time-expansion detector system. I have been using heterodyne and frequency-division detector combinations for observing and recording bat calls. I find that high-quality open earphones (with a high dynamic range) allow me to hear those sounds that are below the frequency window of the detector (social calls), and thus enable me to gather more data about bat behavior. Bats can be observed with the naked eye or in combination with spotlights and night-vision devices.

It is important to remember that in the field, the actual observation of a flying bat and the parameters we can obtain (e.g., acoustic, visual) are usually only a sample of what might be available. Observers learn through experience to recognize species or species groups using a combination of characters (Fig. 1). The same process allows them to recognize the situations in which they cannot identify the bat in question. In the field, it is not always possible to work with exact parameters, making experience with a range of species and situations particularly important in the identification of flying bats.

In my experience, some species are relatively easy to recognize, mostly those hunting in open and semi-open landscapes and using longer, narrowband echolocation calls, with almost or quasi-constant frequency (QCF), e.g., *Nyctalus*, *Eptesicus* and *Pipistrellus* species. Rhinolophids hunting in clutter and using calls dominated by one frequency (real constant-frequency, CF, calls) again are relatively easy to identify. More difficult to identify are those species hunting near vegetation or in clutter and using steep frequency-modulated (FM) pulses. Species identification in general becomes more difficult in cluttered habitats (typically close to or within vegetation) because of convergence in signal design and flight behavior in these situations. The actual bat fauna at a study site (number of species, number of 'similar' species) also determines the degree of difficulty experienced in identification.

In general, positive identification requires a combination of acoustic and visual information, but for some species in some situations where parameters are clearly specific and discriminating, sound or image alone may suffice. I find that in general, a time expansion and heterodyne combination provides the best information for species identification. However, for species and situations where parameters produced by a heterodyne detector are clearly specific and discriminating, that output alone is sufficient.

Identification should always begin in the field based on what is seen and heard. Analysis of recorded sound in the field or in the laboratory allows measurement and quantification of certain parameters, and adds to the process of interpretation and learning. In difficult cases, verifying species identification by analyzing and quantifying recorded calls is of limited use without field observations of the bats in question. For species and situations where parameters are clearly specific, experience makes identification in the field possible with no further analysis. It is good practice, however, to make sample recordings for analysis and documentation. Time expansion and frequency division provide the possibility to analyze the frequency/time domain of individual pulses. A heterodyne system provides excellent data on pulse rates.

PRECONDITIONS, LEARNING AND VERIFICATION

For identification based on sound and flight behavior in the field, as well as through analysis of recorded sound, we not only need to identify and describe species-specific parameters, we also need to study how echolocation and flight work, to be able to relate the behavioral adaptation of sound and flight to the habitat structure (Fig. 2). This is an ongoing process. The derived diagnostic parameters that can be used for identification are somewhat specific to the different detector systems (Limpens and McCracken, this volume).

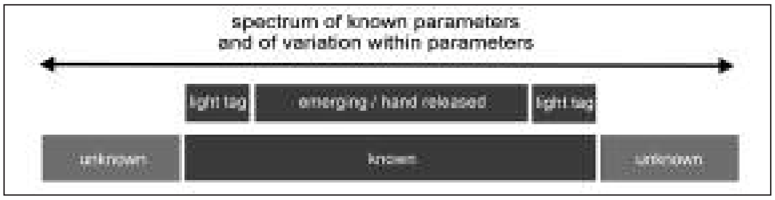

Figure 2: Fundamental description: as a precondition for identification of bats based on their echolocation and flight behavior, specific parameters from known bats (emerging, hand released, marked) must be described. In this ongoing process, there is a need to understand the behavioral variation within and between species.

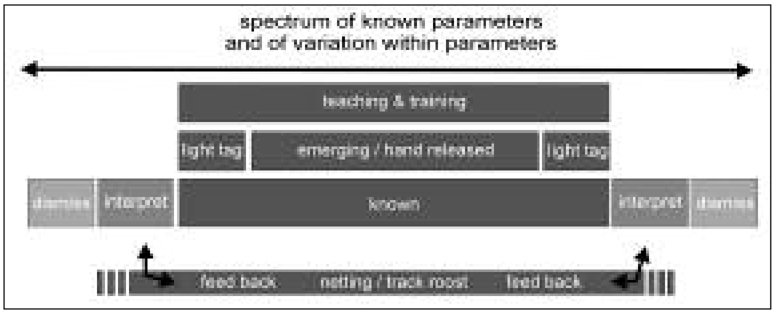

Figure 3: During the learning process, knowledge increases through repeated observations of known bats (emerging, hand released, marked). Species should be studied in as many different behavioural situations as possible. Interpretation of unknown parameters can be handled as a working hypothesis. Feedback on this identification is achieved by netting or tracking bats back to roosts.

Assessing which parameters are species specific depends on recording marked bats of known species (e.g., bats emerging from roosts). These parameters may include bandwidth, frequency in the narrowband ending of a pulse (QCF), pulse length, or repetition rate.

Novice observers learning to recognize and use detectors in the field need to be trained by experienced observers. Repeated study, practice, and development of observational skills (again relating to marked bats of known species identity) are key methods of gaining experience (Fig. 3). To describe a bat's 'vocabulary' and flight, as well as to learn to recognize and interpret parameters, emphasis should be put on determining behavioral variation through observation of different hunting situations (Limpens, this volume).

Developing observational skills and learning to recognize parameters may take years, rather like the process of learning to use binoculars and ears to identify birds (e.g., Bibby et al. 1992). While dusk and dawn provide good observation conditions, observational skills should also be developed using a powerful light during true night. In the learning phase, the observer should start with one or just a few distinct but abundant species, then listen and watch them hunting and flying in different situations. When these species are well known, new species can be added to the learning process. The learning process is a stepwise iterative process. It starts with situations where the species is known. The sounds to listen for come when bats are leaving a roost, released from the hand, or flying after being light tagged. These will, however, never be more than a sample of the spectrum and variety of calls a species is capable of. In my experience and opinion, the next step is to use the experience gained from this 'hard evidence' to interpret what we see and hear in other situations. Our identification of a bat in such a new habitat or hunting situation is thus handled as a 'working hypothesis.' Tracking bats to their roost (Limpens 1993), or netting is used to confirm the identification. This enhances the learning process and moves it beyond what was learned from marked bats (Fig. 3).

Regular verification of identification is needed to retain the necessary quality standard. Both beginning and experienced observers should never lose a self-critical attitude regarding identification. Even in situations where identification with a bat detector is routine, identification should be handled as a working hypothesis. Whenever possible or needed, with respect to the distinctiveness of a situation or species, confirmation of identification should be sought using other methods (Fig. 4).

Identification in the field must be repeatable, and where limited distinctiveness requires it, an independent check on identification improves confidence. Analyses of recorded sounds are an important part of the identification operation and enhance the means for identification in the field. Recordings also serve as documentation and provide opportunities to obtain a second opinion (Limpens et al. 1997, Fig. 4).

Depending on the research question, a certainty/uncertainty bandwidth can be developed, defining how observations should be handled. For a distinctive species in a distinctive situation, field identifications should be routine. The same species in a region where it has not been documented requires a sound recording for documentation and identification by other methods. In large-scale surveys, it is not difficult to use observations made in distinctive (mostly uncluttered) situations and discard the observations of a species detected in difficult situations. In contrast, for detailed analyses of hunting behavior and habitat use, species identity must be determined in all (or most) possible habitats.

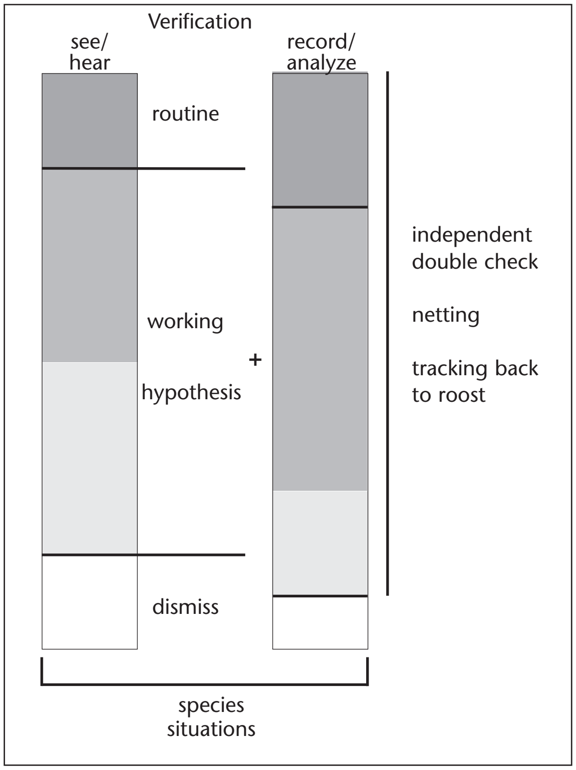

Figure 4: Regular verification through independent second opinion and other methods is required. Recording and analysis are for backup and documentation. The process is dependent on the distinctiveness of species.

PARAMETERS USED FOR IDENTIFICATION

The comprehensive approach I propose uses the recording and all visual and acoustic information available to an observer in the field (Fig. 1). Bat-detector systems that provide visual displays of call information detract from an observer's ability to watch bats while monitoring echolocation calls and behavior. The observed parameters are interpreted in total, not like a dichotomous tree.

Features or parameters of use are found in the bat's: flight image/ appearance:
the size, shape, and color of the body

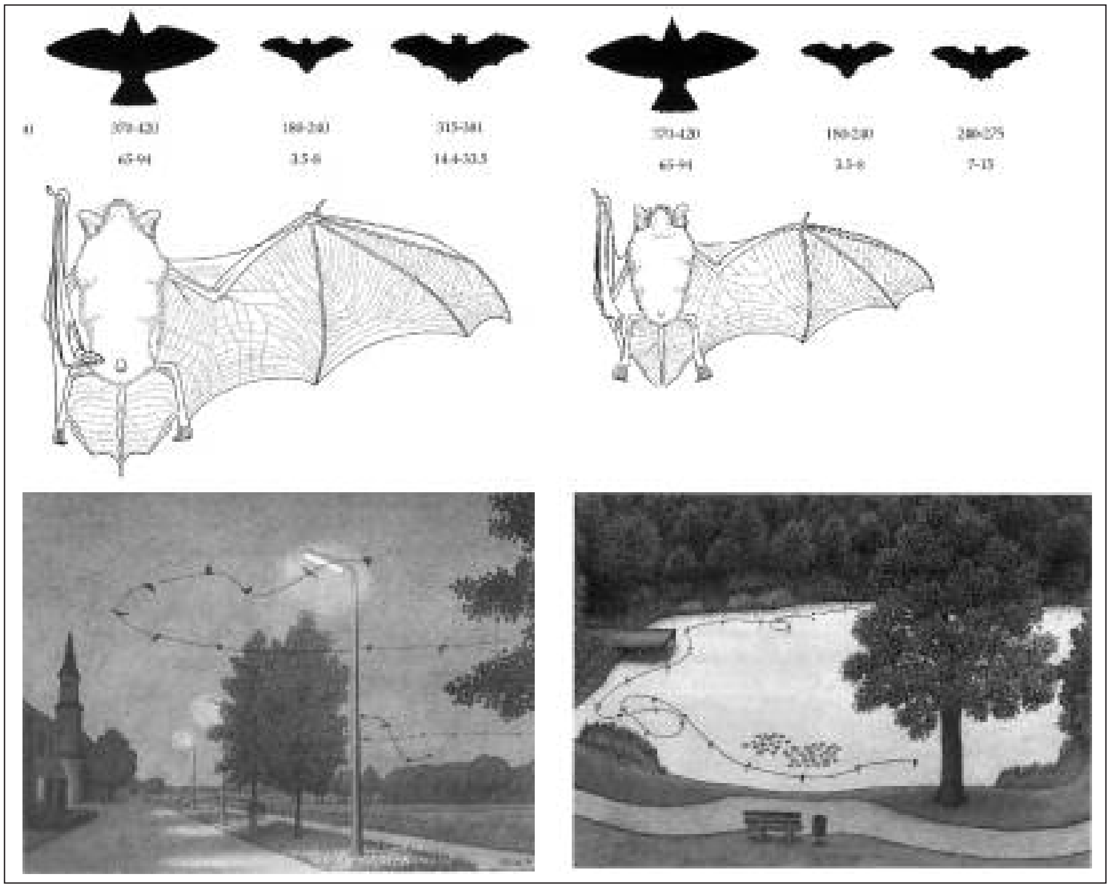

Figure 5: A comparison of *Eptesicus serotinus* and *Myotis daubentonii* to illustrate the differences in size, wing shape, flight image, and behavior between species. For comparison see also the silhouettes of *Sturnus vulgaris* and *Pipistrellus pipistrellus* (after Twisk in Limpens et al. 1997).

and wings; flight style: the species' flight and hunting behavior; sonar or echolocation behavior; social sounds (as produced by flying animals or animals calling from the roost).

Flight Image: Size, Shape, and Color

With practice, and the use of lights, a headlamp, or possibly night-vision equipment, the flying bat and/or its silhouette against the sky can be observed. Table 1 presents features that, with experience, can be used for identification.

For example, a noctule (*Nyctalus noctula*) is clearly larger than a pipistrelle (*Pipistrellus pipistrellus*). A pipistrelle and a whiskered bat (*Myotis mystacinus*) are of similar size, but a pipistrelle has long, narrow wings and a torpedo-like body, relative to the short wings and more rounded body of a flying whiskered bat. *Myotis* species in general have a light dorsal surface, whereas most other European bats are uniformly colored. The long ears of a long-eared bat (*Plecotus sp.*) are visibly larger then those of natterer's bat (*M. nattereri*). Natterer's bat's wings are broader than those of Daubenton's bats (*M. daubentoni*). These details can be obtained from the literature, and reinforced by handling bats (Figs. 1 and 5).

large animal	small animal
torpedo-like body	more rounded body
long wings	short wings
larger wingspan	shorter wingspan
broad wings	narrow wings
(longer) pointed wings	(shorter) rounded wingtips
large / long ears	small ears
v-shaped tail membrane	curved tail membrane
free last tail vertebra	
free tail	
contrast between back and belly	even coloration
light dorsal surface	

Table 1: Features of flight image (size, shape, and color of body and wings), which, with experience, can be observed and used for identification.

Flight Style: Flight and Hunting Behavior

There are a variety of features regarding the flight style, or the flight and hunting behavior of bats, which, with experience, can be observed and used to aid in identification (Table 2).

For example, the large serotine (*Eptesicus serotinus*) flying at 5-10 m, has a relatively shallow wing beat compared with that of the Noctule, which typically flies at higher altitudes. A long-eared bat may sail slowly past the observer, whereas a natterer's bat swoops relatively fast close to and through openings in the vegetation. A whiskered bat may patrol in long and repetitive loops parallel to the vegetation. A nathusius' pipistrelle (*P. nathusii*) often passes in long, straight loops, whereas the common pipistrelle typically dances past in a hectic flight with many curves and dashes in its flight path (Figs. 1 and 5).

deep wing beat	shallow wing beat
fast flight	slow flight
straight flight	curved flight
flight in long loops or straight lines	flight in small circles
flight (almost) in one plane	three-dimensional flight
flight close to water surface	
regular or stereotyped flight	hectic flight
flight in relatively large and slow curves	sudden changes of direction
flight in wide curves	narrow curves
steep dives	slow and shallow dives
flight high in the air	low flight height
flight in open area	flight close to vegetation
flight parallel to vegetation	following the surface structure
	flying through the vegetation
	hovering
	hanging from branches: perch hunting
	hanging from stem: perch hunting
	crawling on stem
	landing / crawling on ground

Table 2: Features of flight style and hunting behavior of bats, which, with experience, can be used to aid identification.

Sonar or Echolocation Behavior

Complementing visual observations, echolocation calls heard from the detector or with the unaided ear and processed in sound analysis provide numerous parameters useful for identification. Limpens and McCracken (this volume [Table 2]) describe how different detector systems reveal different parameters.

Echolocating bats use two basic signal types, with different functional properties: constant-frequency signals (CF) and frequency-modulated signals (FM). CF bats emit a relatively long (often 50 ms and longer), pure tone signal on a constant frequency. In contrast, the calls of FM bats generally start at a higher frequency (e.g., 70 kHz) and sweep down in a relatively short time (1-5 ms) to a lower frequency (e.g., 25 kHz). In Europe, the Rhinolophidae are CF bats, and the Vespertilionidae and

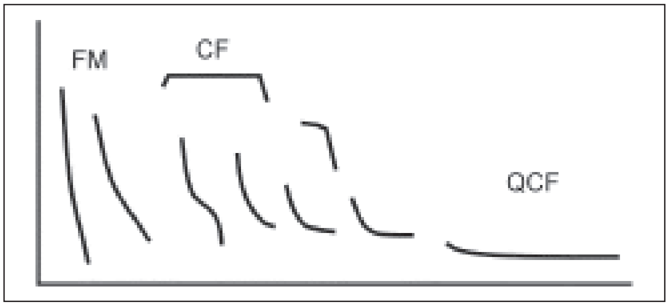

Figure 6: Schematic diagram of different call designs in European species. With constant frequency calls (CF) in the Rhinolophidae, and steeper frequency modulated calls (FM) to narrowband calls (quasi-constant-frequency QCF) in the Vespertilionidae and Molossidae.

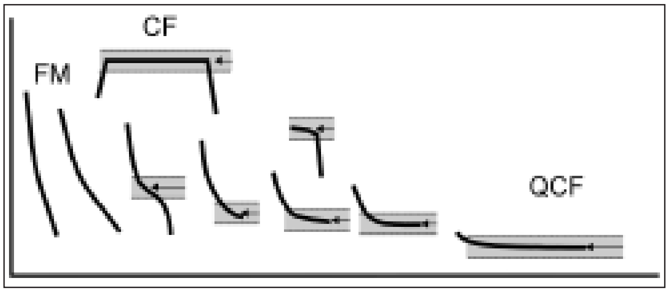

Figure 7: Tonal quality in a pulse is audible when the frequency change of time (FM rate) is slow enough: in CF, shallow FM or narrowband parts of a pulse.

Mollosidae are FM bats (Fig. 6).

In reality, the echolocation calls of a horseshoe bat start at a relatively low frequency, sweep up to the constant frequency, and end with a downward sweep. Thus the call in fact has three components: 'FM-CF-FM.' Some FM bats produce calls that show sharp changes in frequency (large bandwidth) over short durations (2-4 ms). These are true broadband echolocation calls. Others show initial rapid (1- 2 ms) changes in frequency over time but end (4-15 ms) in more gradual changes. These are often comparatively narrowband pulses. The shallow part toward the end of such a pulse can be *almost* constant in frequency, or Q*uasi* Constant Frequency. This is still an FM pulse, functionally different from a CF pulse (Boonman 2001), but the end part might be called QCF, with a sweep rate < 1 kHz/ms (Fig. 6, e.g., Fenton 1999; Kalko and Schnitzler 1993; Limpens and Roschen 1995).

Frequency-Related Parameters

Tonal Quality

In the field, the presence or absence of so-called tonal quality, related to the bandwidth of FM calls and pulse shape (pattern of frequency change over time), is a primary parameter used to distinguish between species or groups of species (Ahlén 1981; Helmer et al. 1987; Limpens and Hollander 1992; Limpens and Roschen 1995). For humans to perceive the tone or pitch of a sound, we need to hear each frequency for a minimum duration. When the frequency in a call changes rapidly over time, the tonal quality of the signal will be less obvious than when the change in frequency is slow. Tonal quality is detectable in the CF, or narrowband, parts of a pulse (Fig. 7). A steep FM pulse is heard as a 'dry' tick without tonal quality.

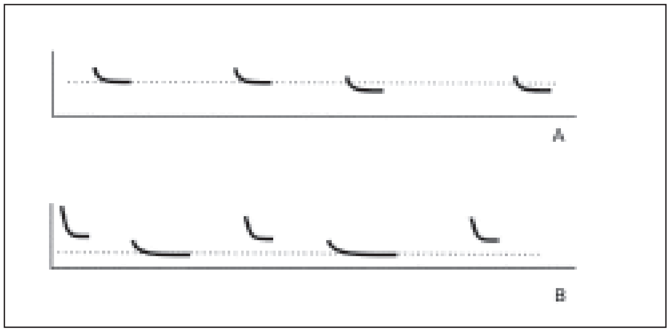

Figure 8A: When tonal quality is audible in a passing bat, the Doppler shift in the frequency as compared to the tuned frequency can be heard as change of pitch in the output of a heterodyne detector. **8B:** When tonal quality is audible, alternating use of different pulse types by a Noctule (*Nyctalus noctula*) is clearly audible in the alternating pitch.

Using a time-expansion system, our ears can more readily recognize tonal qualities in signals. When tonal quality in the output is audible, other qualities can also often be detected. For example, the Doppler effect in the output signal of a heterodyne detector is a result of the bat's flight speed. In combination with the duration of the bat pass, this change in output conveys information about flight style (speed and curvature; Fig. 8A). The use of alternating pulse types with different structures, in terms of frequency range, FM rate, and length of the narrowband part of the call, is clearly audible when the bat is using pulses with tonal qualities. For example, a noctule flying at high altitude alternates pulses with different bandwidths, producing an alternating 'pjiep' 'pjoup' sound on a heterodyne detector (Fig. 8B).

The true CF part in the pulse of a horseshoe bat is perceived as a clear whistling sound. Differences between calls produced by stationary or flying bats and straight or curved flight are obvious from the changes in pitch produced by Doppler shifts in these long pulses.

Best Listening Frequency: Even Distribution of Energy

In descriptions of bat sounds, a certain (narrow) frequency band in the signal is often referred to as the 'best listening frequency' or sometimes 'peak frequency.' To find the best listening frequency using a heterodyne detector, I tune the detector to get the lowest possible pitch in the output (Fig. 9). The best listening frequency often coincides with the shallow FM or the narrowband part (QCF) of a pulse (Fig. 7).

Gleaning species, which use steep broadband FM signals, but also other species when they fly in cluttered environments, use a relatively even distribution of energy over the whole frequency range. There is no clear best listening frequency. The absence of a best listening frequency is an indicator of an even distribution of energy throughout the call (Fig. 9).

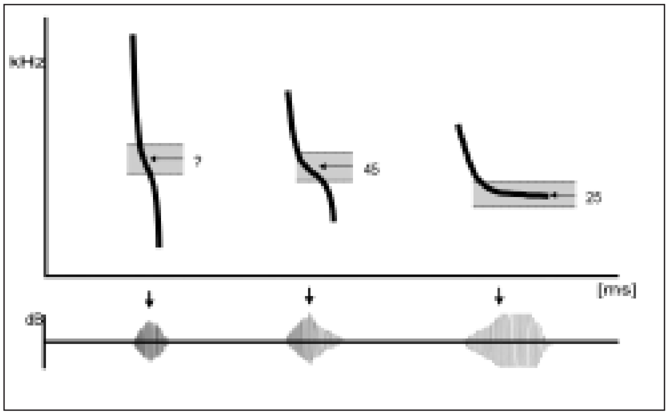

Figure 9: The best listening frequency often coincides with the shallower part, or the narrowband (QCF) part of a pulse. An even distribution of energy, making for no best listening frequency, is also diagnostic.

Harmonics

Harmonics occur as a component of the bat's signal or even as artifacts of signal processing in the detector or analysis software. Harmonics can be analyzed using a time-expansion system and can be heard in the field with heterodyne detectors. Use of harmonics for species identification, however, requires more research. In species where the discriminating narrowband QCF frequencies are relatively close (e.g., *Eptesicus serotinus* versus *E. nilssonii*, or *Pipistrellus pipistrellus*, *P. nathusii*, and *P. kuhlii*), an analysis of harmonics, where the 'gap' between harmonics is doubled or tripled, can enhance discrimination (Fig. 10).

Frequency Range

Bat signals are often described with respect to the range of frequencies in a call, which can be expressed as bandwidth. Thus, a call sweeping from 80 to 40 kHz has a bandwidth of 40 kHz. Because atmospheric attenuation is stronger at higher frequencies (e.g., Griffin 1971; Lawrence and Simmons 1982), the distance between bat and microphone influences the presence of high frequencies in the received signal, resulting in increased variation in, and often underestimation of, maximum frequency. The directionality of the microphone and the 'beam of sound' produced by the bat are also frequency-dependent. The position and angle of flight direction of the bat relative to the plane of the microphone membrane therefore influences the presence of high frequencies in the received signal. As a result, maximum frequency is a difficult parameter to measure in the field or in the analysis of recordings of free-flying bats. The lowest frequency is less affected by these phenomena. In narrowband pulses, lowest frequency almost always coincides with the best listening frequency in the QCF part of the call (Fig. 6).

Shape or Curvature

The pattern of change in the FM rate or the presence of real CF, such as in a horseshoe bat's pulse, gives pulses a specific character or shape. Species exhibit a range of possible shapes (Fig. 11) and individuals show flexibility in relation to the hunting environment and behavior. FM pulses can be almost linear (e.g., *M. bechsteinii*, *M. nattereri*, *M. myotis*), curvilinear (e.g., *Plecotus* spp., *M. mystacinus/brandtii*), or bilinear, with a steep, even portion followed by a shallower part, terminating in a further rapid sweep (e.g., *M. daubentoni*, *M. mystacinus/brandtii* in open habitat, *M. myotis* in open habitat). Curvilinear calls may have a relatively long narrowband QCF ending (e.g. *Pipistrellus* sp., *Nyctalus* sp., *Vespertilio murinus*, *Miniopterus schreibersii*). Bilinear calls may appear as steeper FM, with a clear angle to the narrowband ending of the pulse (*Eptesicus* sp.). In relatively open habitat situations or while commuting, the shallow part in the middle of the pulse of, e.g., *M. dasycneme* or *M. myotis/blythii* may become very narrowband (QCF). In open situations, the narrowband pulse of, e.g., *N. noctula* may lose its steeper FM beginning altogether, while the pulse of a species like *M. dasycneme* loses the steeper FM parts at the beginning and end of the pulse. Distinct patterns include the hook-shaped QCF-FM calls of the barbastelle (*Barbastella barbastellus*), beginning with a shallow part and ending in steeper FM, or the FM-CF-FM calls of the Rhinolopidae (Fig. 11 – e.g., Ahlén 1981; Kalko and Schnitzler 1993; Limpens and Roschen 1995; Limpens et al. 1997; O'Farrell et al. 1999; Tupinier 1996; Weid and von Helversen 1987).

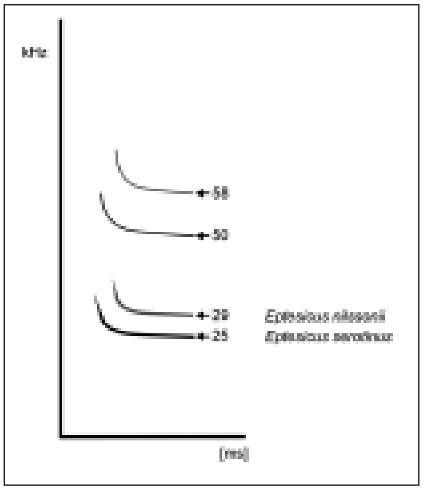

Figure 10: Harmonics can help differentiate between similar best-listening frequencies.

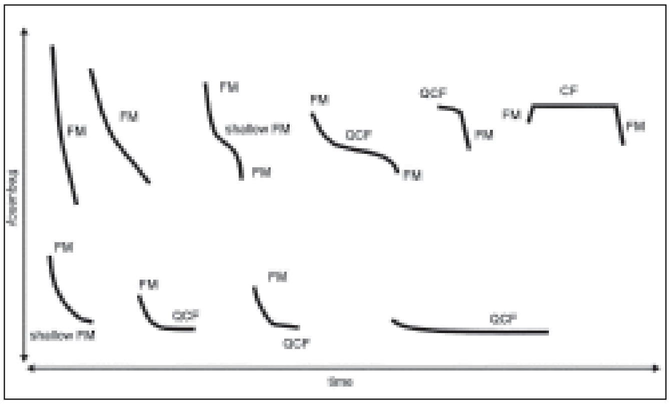

Figure 11: Examples of pulse shape.

AMPLITUDE-RELATED PARAMETERS

Loudness: Maximum Sound Pressure Level

While some bats produce intense echolocation calls (>110 dB SPL @ 10 cm), others produce much less intense calls (60 dB SPL @10 cm; Fig 11). Intense calls are readily detectable at greater distances (> 10 m) than quieter calls (< 1 m). Gleaning bats often use low intensity calls, making them much less conspicuous to even the most sensitive bat detectors. To interpret large differences in the field or in analysis of recordings, the influence of attenuation and microphone sensitivity must be accounted for in relation to the distance and angle of observation of the observed bat.

Since bat pulses are very short sounds to our ears, somewhat longer pulses of equal amplitude, containing more total sound energy, might be perceived as louder (Fig. 12).

TIME-RELATED PARAMETERS

Pulse Length, Interval Length, and Repetition Rate

Bat signals can be described with respect to the duration of pulses and inter-pulse intervals, which together determine the repetition rate.

Recordings from time-expansion and frequency-division detectors are useful for accurate measurement of these features, whereas heterodyne detectors allow accurate measurement of pulse rates (Figs. 13a and 13b). In the field, pulse length and interpulse intervals are perceived as longer or shorter relative to other species. The pulse rate can be perceived as being slower or faster. It is impossible to measure a precise value, or distinguish between species close to each other, but it is possible to reliably distinguish between species with large differences, or to recognize that, in the observed pulses and pulse train, these features differ from those of a known reference species (Ahlén 1981; Ahlén and Baagøe 1999; Limpens and Roschen 1995).

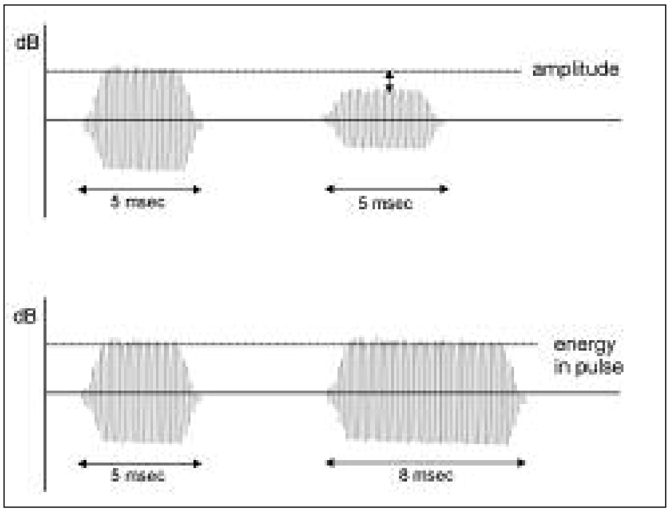

Figure 12: Pulse intensity differences can be determined by differences in amplitude (real sound pressure) or differences in energy in a somewhat longer pulse.

Rhythm

Pulse rate is related to the wing-beat rhythm (typically one pulse per wing beat), which is related to the size of the bat, its wing shape, wing loading, habitat, and the actual behavior such as hunting, commuting, or swarming.

Some species, such as *Eptesicus serotinus* or *Pipistrellus nathusii*, in some flight situations, commonly omit a pulse in the normal repetition of pulses, and pulse intervals tend to be variable. This produces specific irregularities in their repetition rate: a rhythm (Fig. 13c). Some species, such as *Nyctalus noctula*, alternate differently structured pulses, with a different tonal quality, and differences in the length of the pulses and the following interval, again resulting in a characteristic rhythm (Figs. 13d and 13e – Ahlén 1981; Limpens and Roschen 1995; Limpens et al. 1997).

SOCIAL SOUNDS

Bats use a repertoire of social calls or sounds in display flights or while calling from roosts. The lower frequency ranges of these sounds are readily audible to human observers. These communication sounds can be species-specific. However, the time-frequency structure of social calls is much more complex than that of sonar signals. As a result, bat social sounds are not always easy to interpret.

Unlike sonar signals, social calls are irregular and occur in specific circumstances, such as during social interactions, display flights in a mating territory, when calling from a mating roost, before emergence, or after entering a roost. In some species, social sounds in flight are seldom heard, so it is difficult to learn what sound is linked to what species. Nevertheless, in all cases where social sounds can be attributed to a specific species, they provide an excellent additional feature for field identification or through analysis of recordings (Fig. 14 – e.g., Ahlén 1981; Limpens 1993; Limpens and Roschen 1995).

SYNTHESIS AND INTERPRETATION

To collect as much information as possible, observers must be willing to spend time watching and listening to an individual species, or the species in an area, until the distinctive combination of parameters is observed. Typically, it is best to select a habitat or environment situation in which certain species are known to be distinctive (Ahlén 1980b, 1990, 1993; Limpens et al. 1997). The overall picture that results relies on combinations of visual and aural information including: impressions on size, shape, and color of the whole animal; wing and ear shape and size; flight style or flight behavior in relation to habitat structure; aural observations regarding tonal quality, pitch, FM-rate, Doppler effect in the output, alternation of pulse types, best listening frequency, the frequency of the narrowband (QCF) ending, distribution of energy, presence of harmonics, frequency range/bandwidth, F_{max} / F_{min}, loudness, pulse length, interval length, repetition rate, and, last but not least, rhythm. Taken together, these cues will allow reliable identification to the species level, or a group of 2-3 similar species in most cases (Ahlén 1990; Ahlén and Baagøe 1999; Limpens and Roschen 1995; Limpens et al. 1997).

The observer can compare aural and visual parameters with his or her own sound and image memory. Knowing and visualizing the basic principles of pulse design and of flight in relation to wing and body morphology enhances the ability to observe and interpret parameters (Fig. 1). When interpreting this mixture of cues, most species can be ruled out immediately. The parameters that are most important for discriminating between the two or three species left to be compared depends on the species involved.

Examples

I deliberately position myself on an open, uncluttered part of a road between a small village and a wooded hilltop. Here I expect to have the best view of passing bats and hear the most distinctive sounds. At dusk, I observe a large bat flying towards the hilltop. I can see that it is larger than *E. serotinus* and has broader wings. The heterodyne detector tuned to 40 to 45 kHz does not produce either the metallic tonal output associated with the harmonic of the best listening frequency for a serotine, nor its typical galloping rhythm. Rather the output is a slow, rather regular, and relatively loud rhythm like a dry 'tick tick tick tick ...'. I record the bat pass in the memory of the time-expansion detector. The next bat flying by gives me the chance to tune down to between 30 and 35 kHz. At that frequency, the bat's call produces a short, smacking tonal quality, like a regular 'tack tack tack tack ....'. In the beam of my light, I see a white belly and, in the silhouette against the sky, a V-shaped tail membrane, lacking the free last vertebrate to be expected in *E. serotinus*. These aural and visual cues together tell me that these must be mouse-eared bats (*Myotis myotis* or *Myotis blythi*; a species pair not able to be separated in flight). Later analysis of my time-expansion recordings confirms this identification. The calls are relatively long (up to 10 ms), FM pulses with a more narrowband portion between 30 and 35 kHz (e.g., Limpens and Roschen 1995; Limpens et al. 1997). Following the bats' flight path back to the village in the morning, or checking larger attics, might allow me to find the roost and refine my identification as to whether they are *M. myotis* or *M. blythii* (Limpens 1993; Limpens and Roschen 1996, 2002).

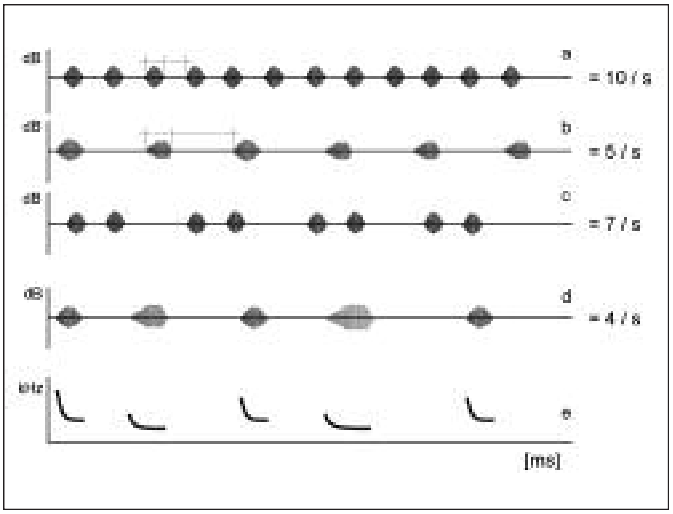

Figure 13 a and b: The length of the pulses and the interpulse intervals together represent the repetition rate, which differs between species. **c-e:** Irregularities in the pulse emission and use of alternating pulse types result in characteristic rhythms.

In another situation, I choose a spot on a wooded riverbank where I can silhouette bats against the dusk sky. Among others, *M. daubentoni* start hunting in their typical flight style, taking insects from the water with their hind feet. Their flight behavior, white bellies, and longer and broader wings distinguish them from pipistrelles. They produce a fast rhythm of dry 'ticks,' with a best listening frequency of 40-45 kHz. The pulse repetition rate is variable, but increases and decreases in a regular way. Later in the evening, other *Myotis* start to hunt higher up and parallel to the vegetation on the bank. Is this a different *Myotis* species, or are these still Daubenton's bats? These bats have a white belly, too, but seem a little smaller; their wings are shorter and their bodies more compact and round. Again a dry rhythm with a best listening frequency of 40-45 kHz, but the rhythm is slower and quite regular, and lacking the two-stroke rhythm Daubenton's bats can have when flying in long loops. My working hypothesis is that these are *Myotis mystacinus/brandtii.* Again these bats are sibling species that are difficult to identify to the species level, based on sound and flight behavior.

Analysis of time-expansion recordings show similar pulse designs for both recorded Myotis species: moderately steep, somewhat sinusoidal FM pulses, from about 70 to 25 kHz, with a little shallow FM at 45 kHz. Larger and more regular pulse intervals can be observed in the latter species group. The Daubenton's pulses show a typical knotted form, on the spectrogram as well the oscillogram, due to the interference between signal and echo from the water surface (Kalko and Schnitzler 1989). Especially when the recordings of whiskered/Brandt's bats are from individuals flying farther away from the vegetation, this distortion is not evident. This is not diagnostic, however, since a whiskered bat flying down to the water to drink would give the same artifact.

At dawn, I observe a concentration of these 'whiskered/Brandt's bats' again on a bank near the village. I follow them to their roost and find them swarming at a wood-covered wall, making turns of up to 4-5 m. Some other white-bellied, somewhat larger bats with broad wings and larger ears are also swarming at the exact same spot. They are flying slower and much closer (< 1 m) to the wall. The overall call rhythm of the swarming bats is a clutter of fast, dry ticking, from which it is impossible to make out individuals or species. The belly of the new species is too white and their ears are too small to be long-eared bats (*Plecotus* sp.). They must be *Myotis nat-*

tereri. Hand netting on the following evening reveals pregnant *M. mystacinus* and pregnant *M. nattereri*. We have identified the bats to species, found their roost, flight path, and hunting sites and determined their reproductive status and sex.

ACKNOWLEDGEMENTS

I thank the organizers of the symposium for providing the opportunity for such an intensive exchange and discussion, as well as those who read and commented on earlier versions of this manuscript, including: Ingemar Ahlén, Lothar Bach, Michel Barataud, Brock Fenton, and Gary McCracken. I thank the reviewers and editors for their help with the manuscript, and Peter Twisk for his beautiful illustration of the 'puzzle of identification' in the field, as well as his comments on the text.

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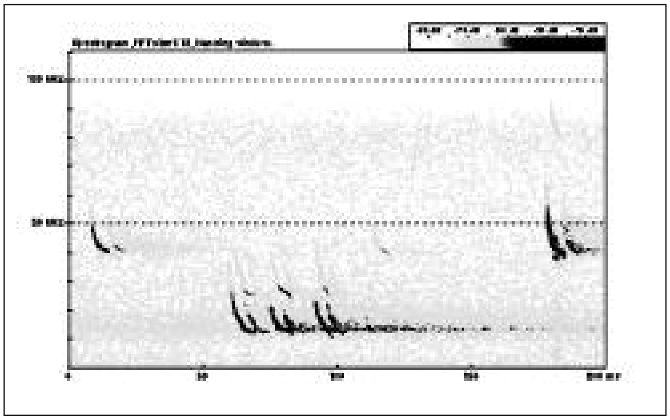

Figure 14: Echolocation calls and the social sounds produced by male *Pipistrellus kuhlii* in display flight.

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ACOUSTIC SURVEYS AND NON-PHYLLOSTOMID NEOTROPICAL BATS: HOW EFFECTIVE ARE THEY?

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In the Neotropics, distributions of members of the family Phyllostomidae, or leaf-nosed bats, are relatively well known. Traditionally they have been sampled using mist nets. Distributions of the other eight families occurring in the Neotropics are poorly known. In general, species in these families are insectivores with well-developed echolocation abilities. In most cases, this allows them to detect and avoid mist nets, although harp traps are an additional sampling tool. Acoustic survey methods have provided significant contributions to the current knowledge of the distribution of these species, although these surveys are not suitable for all species. Acoustic identification in the field of Emballonuridae (sac-winged bats), Noctilionidae (fishing bats), Mormoopidae (leaf-chinned and moustached bats), Vespertilionidae (evening bats) and many Molossidae (free-tailed bats) is relatively straightforward. However, it appears that acoustic methods are not yet suitable to identify Natalidae (funnel-eared bats) or Thyropteridae (disk-winged bats). The calls of *Natalus stramineus* and *Thyroptera tricolor* are too quiet to be detected during acoustic surveys. Large sample sizes of call sequences, as well as viewing calls in real time, are necessary to allow a complete understanding of variation in the search-phase calls of a given species. Details about echolocation calls recorded using a time-expansion system and visualized with spectral analysis, such as harmonics and amplitude, do not contribute significant information to enhance identification of Neotropical species beyond what is currently available using the Anabat suite of hardware and software. Apparent call variability within the Vespertilionid genus *Myotis* and several species of the Molossidae may be due to unresolved taxonomic issues or incorrectly identified species rather than geographic variation. In the Neotropics, only vocalizations confirmed by vouchers can reliably be used as reference calls to make positive identifications.

Key words: acoustic identification, Anabat, Neotropical bats, spectral analysis, time expansion, zero-crossings analysis

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Distributions of species of Phyllostomidae, or leaf-nosed bats, are relatively well known in the Neotropics. They are, for the most part, readily captured with mist nets. In contrast, the other eight families occurring in the Neotropics are less well known. These insectivores have well-developed echolocation abilities allowing them to detect and avoid mist nets. Although harp traps have been used for sampling, acoustic survey methods have provided significant recent contributions to the knowledge about distributions of these species.

During the past six years, I have used two acoustical systems to record bat calls in the Neotropics (primarily Belize), Anabat and Pettersson D980. The Anabat system uses frequency division and zero-crossings analysis (ZCA) and the Anabat suite of analytical software. Although the Pettersson detector also includes heterodyne and zero-crossings modes, only the time-expansion mode was used. Recordings were analyzed using spectral analysis in BatSound Pro software. Each system has its own inherent strengths and weaknesses (see other chapters in this volume). It is possible to use call shapes and frequencies recorded in one system to identify species recorded in another. However, it is important that comparisons of call parameters used to describe the range of variation of a species from field recordings be made with similar systems.

Bat detectors and relevant analytical software should be viewed simply as a set of tools used to answer specific research questions. An important first step is to formulate the research question before selecting the tools needed to generate the relevant data. For the present study, the goal was simply to use acoustics as a means to identify free-flying bats and not to examine details of echolocation call parameters per se.

Bat-detector systems were designed for different purposes (heterodyne, time expansion, and frequency division), and it may be difficult to objectively compare them. The literature is full of acrimonious debate about the merits of one system relative to another. Many of these papers are dogmatic and/or polemical; with statements being made based more on opinion rather than supported by fact. Parsons et al. (2000) provides a summary of techniques used for analyzing and transforming bat calls.

Much of the debate has centered on harmonics and the fact that one system detects and displays many of them simultaneously, while it is implied that the other cannot display or deal with harmonics at all. It is important to briefly address some misconceptions about harmonics and ZCA as used in the Anabat system as related to Neotropical bats.

For the researcher new to acoustic identification of Neotropical bats, perhaps the most perplexing papers in this debate have been Fenton et al. (1999, 2001). It is difficult to understand why the authors assumed that variation in harmonic content would be confusing or believed that the Anabat system would not be reliable or useful in identifying common species such as *Rhynchonycteris naso* (Fenton et al. 2001). Perhaps it can be attributed to their small sample sizes. The first sequence of calls recorded with the Anabat was reported to be dominated by 100 kHz pulses (n=12 pulses) and the second sequence's pulses were in the 98 kHz range (n=15). Perhaps it could have been attributed to attempting to extract measurement parameters from the Anabat V recording software (Fenton et al. 1999) rather than using Analook software to analyze calls.

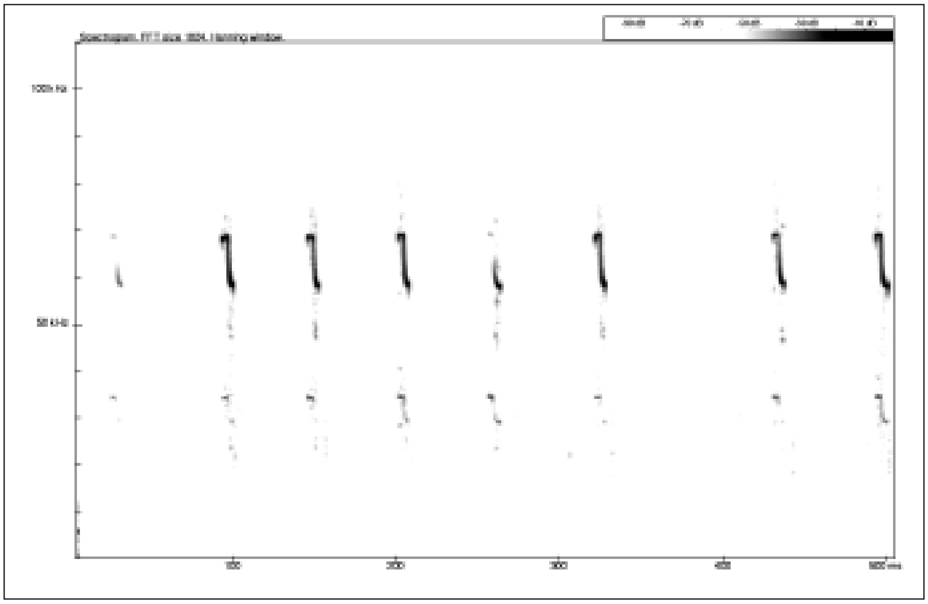

Figure 2: Harmonic display of *Pteronotus davyi* showing the fundamental frequency at the lower scale and first and dominant harmonic on upper scale recorded using time expansion and displayed using spectral analysis (Pettersson D980 and BatSoundPro).

Fenton et al. (1999) placed much importance on the "missing harmonic content" in Anabat recordings, but they failed to detect the second harmonic in three of the five sequences using the time-expansion system. Few pulses were recorded in these sequences (n=3-10). Because full call sequences were not presented for either sampling method, it was not possible to determine if the pulse parameters recorded from the eight individuals that were leaving a roost and flying within a boathouse (Fenton et al. 1999) were representative of search-phase echolocation calls for the species.

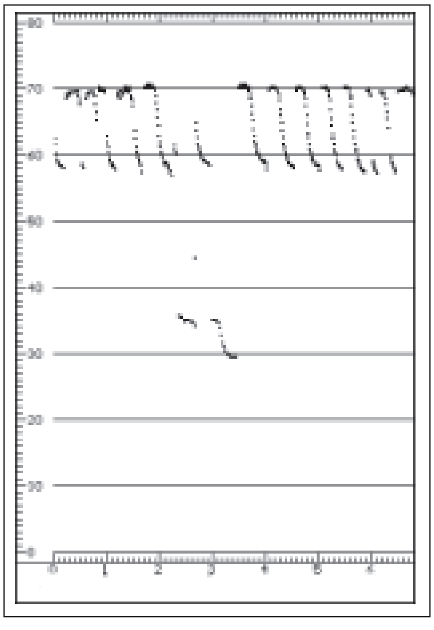

Figure 1: Harmonic display of *Pteronotus davyi* showing the fundamental frequency at the lower scale and first or dominant harmonic on upper scale recorded with frequency division and displayed using zero-crossings analysis (Anabat system).

I suspect that under such conditions, recordings made by either method would result in the high clutter calls *R. naso* routinely emits when leaving roost sites (O'Farrell and Miller 1997). These are quite different and not representative of the search-phase calls of free-flying bats and would certainly be confusing to use for identification purposes.

An important point to clarify is that the Anabat detector is a separate component of the system and as such it detects all ultrasonic sounds within the broadband range of 10 to 200 kHz. It is also true that ZCA only responds to the loudest or dominant component of the incoming signal from the detector as it is processed (Corben, this volume). If more than one harmonic is present in the original signal, only one of these will be displayed (Fig. 1). The loudest harmonic corresponds to those pulses with the maximum energy when examined with spectral analysis where both the fundamental and first harmonic are simultaneously displayed (Fig. 2).

Clearly, the Anabat system can and does record the multiple harmonics of *R. naso* (O'Farrell and Miller 1999), as well as those of many other species. Based on a large number of calls (N>600) recorded from many individuals (N>30) from widely separated locations, I have seen harmonic shifts in calls of *R. naso.* Which harmonic is displayed is related to the intensity of calls at the time of recording. For example, when recording with the Anabat system, the second harmonic is frequently the loudest one reaching the detector and therefore is displayed most often (Fig. 3a). In the case of *R. naso*, it appears that when the fourth harmonic is strongest, it will be displayed (Fig. 3b). Higher frequencies attenuate more rapidly and are not detected at great distances. The lack of a simultaneous display of multiple harmonics when using ZCA has not precluded identification of *R. naso* or other Neotropical bats to date.

A critical point is that small sample sizes fail to provide a sufficient range of variation for a given species. Additionally, fragments of calls are inadequate for identification.

When comparing recorded echolocation calls of common Neotropical species using time expansion and spectral analysis with frequency division and ZCA, it was clear that the time-expansion system provided con-

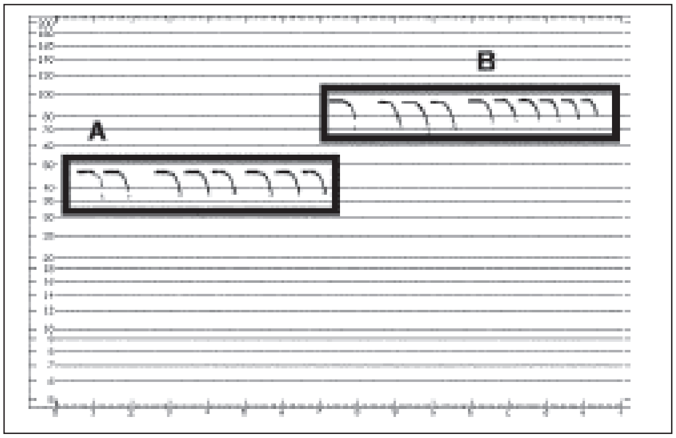

Figure 3: Harmonics of *Rhynchonycteris naso* frequently seen when recording with the Anabat system: **A:** the second harmonic; **B:** the fourth harmonic.

siderably more detail for a given call sequence. Details on amplitude and the display of simultaneous harmonic content were available that were not possible to display using the frequency division and ZCA system.

However, even with this additional information, I found no details that enhanced identification of known bat species. My experience has shown that identifying the non-phyllostomids in the Neotropics requires neither amplitude nor display of multiple harmonic details. However, these details may prove important for identifying species of the family Phyllostomidae (Kalko, this volume).

This raises the question of how much detail is necessary to identify Neotropical bats using echolocation calls. Some authors advocate a qualitative method of identifying bats based upon call pulse shapes and key pulse parameters such as minimum, maximum, and average frequencies, as well as pulse durations (O'Farrell and Miller 1997, 1999; O'Farrell et al. 1999). Others propose quantification based on neural networks or discriminant function analysis to classify bat calls using time expanded and directly sampled echolocation calls (Burnett and Masters 1999; Jones et al. 2000; Parsons and Jones 2000). In these studies, identification rates were reported to be upward of 80% and, in some cases, higher. The call parameters used in these studies were similar to those readily obtained using ZCA and did not include the additional information on harmonics nor amplitude as important characteristics used to identify species during the classification process.

For identifying Neotropical species, it is clear that more detail is better. By this, I do not mean more details from a limited number of calls, but data from more calls, in order to fully understand the range of variation one can expect within a given species.

There was a popular television quiz show broadcast on U.S. television from the late 1950s until the mid-1970s called *Name That Tune*. This game show featured contestants who tried to guess the name of a song, based on as few notes as possible. "I can name that tune in X notes" became part of classic American slang during that time. Although some contestants were quite good at the game, most needed the musical notes to be played in context, to provide a frame of reference. A single note played out of context made it impossible for any contestant to "name that tune." This also holds true when attempting to identify bats by their echolocation calls.

It is critical to recognize that regardless of the recording system used, fragments of pulses or short call sequences out of context often preclude their use for species identification. Experience has proven that just a few notes or "pulses" are usually not enough to "name that bat." It is simply not possible to accurately identify bats from echolocation calls 100% of the time, regardless of the system used to record them.

To illustrate this point, I compared call fragments with complete call sequences for the naked-backed bat (*Pteronotus davyi*) a common Neotropical species (Fig. 4). In this example, if only single short segments (i.e., 3 seconds, which is the memory limitation of the standard Pettersson D980) were recorded and no additional recordings were made, the recorded sequences may not have been suitable to accurately identify the species (Fig. 4). With longer recording sequences, the complete call was seen, allowing unequivocal identification.

It is imperative to have an understanding of the range of variation of a given species' vocal signature for confident identification in the field. To reiterate, more details are needed, but in lieu of increased detail such as amplitude and harmonic content on a limited number of calls, larger sample sizes that provide details of the range of

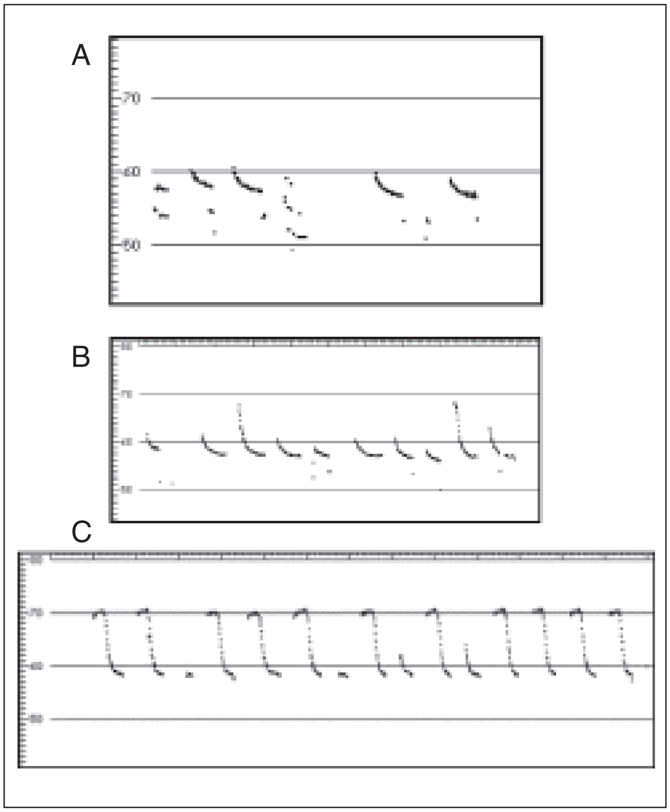

Figure 4: Calls of the common Neotropical bat *Pteronotus davyi*. **A** and **B:** out-of-context fragments; **C:** a full call sequence.

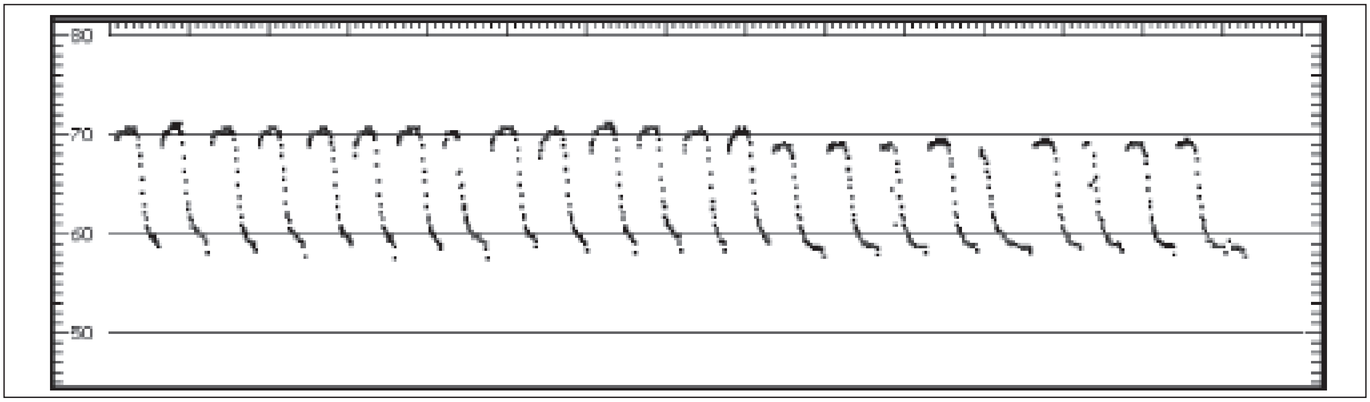

Figure 5: Call sequence showing the frequency shift between two individuals of the same species (*Pteronotus davyi*) foraging in the same space.

variation for key call parameters such as the maximum, minimum, and characteristic frequencies (Fmin, Fmax, Fc), as well as pulse duration (Dur).

Without a large series of recordings for even the most common species, I would not have observed the frequency shift in calls of individuals. This was noted by watching calls on the computer screen in real time with the Anabat system while observing the bats with a spotlight. Frequently an individual shifts the characteristic frequency up or down when another conspecific arrives in the same area. This shift generally ranges from 2-3 kHz. An example of this frequency shift between two individuals of the same species (*P. davyi*) foraging in the same space can be seen in Fig. 5. Observers unaware of such frequency shifts when conspecifics use the same space could overestimate the variation in the basic search-phase calls of an individual or species.

To illustrate this point, I extracted data for call parameters from the recordings (Fig. 5). If only 15 pulses were recorded at the beginning of the call sequence, the range in frequencies would be Fmax=70.8 kHz, Fmin=58.3 kHz, and Fc=67.6 kHz. If only the 11 pulses at the end of the call sequence were recorded, these parameters would be Fmax=68.2 kHz, Fmin=58.0 kHz, and Fc=61.6 kHz. If this sequence were recorded using time expansion, which cannot display bat calls in real time (Pettersson, pers. comm.), researchers may erroneously assume that they were seeing variation in the search-phase calls of one individual.

Like any new endeavor, there must be a starting point. In the beginning, every call sequence recorded is by definition, an "unknown species" acoustically. It was clear there were many different types of calls based on shapes and frequency. The first task was to match faces with voices. This meant bats had to be captured for verification of species identification. Captured individuals were flown under controlled conditions, either in enclosures or in the field, to match recordings to positively identified animals. Chemiluminescent light tags were often used to allow released bats to be followed with the detector.

At present, 28 of the 32 species of the non-phyllostomid bats (7 families) in Belize can be positively identified acoustically (Appendix 1). However, many other species that do not occur in Belize can also be identified acoustically. I have recorded and confirmed by capture dozens of common species that occur in Belize, Mexico, Honduras, Guatemala, El Salvador, Costa Rica, Venezuela, and Bolivia. I have not found geographic variation in the calls of these species to the extent that hinders identification after careful comparison to the Neotropical bat call libraries that are based on verified species calls.

In the Neotropics, it can be both easy and difficult to identify bats using echolocation calls. It has been easy because with the multitude of vastly different families and genera, call sequences often separate readily. It has also been difficult because we do not fully understand the range of species distributions or, in many cases, species limits in the Neotropics.

For example, there has been an increase in the number of recognized species since 1993 from 923 to an estimated 1,095 (Koopman 1993; Simmons, pers. comm.). This is an 18% increase. These taxonomic changes have implications for preconceived geographic ranges. Older published maps and museum identifications may no longer be valid (Simmons, pers. comm.).

For several families (Emballonuridae, Noctilionidae, Mormoopidae), acoustic identification of species appears to be straightforward (O'Farrell and Miller 1997, 1999). Others (e.g., Natalidae, Thyropteridae, Vespertilionidae, Molossidae) are more problematic. As my field techniques were refined and the limits of the equipment established, it was clear that acoustic identification in the field was not an option for species from two families. These were Natalidae, the funnel-eared bats, and Thyropteridae, the disc-winged bats.

The Mexican funnel-eared bat (*Natalus stramineus*) appears to be a gleaner that is too quiet to detect in the field. This species is readily trapped and occasionally mist netted. A large number of fragmented recordings for a few individuals (n=24) were obtained, but only at very close range. Rydell et al. (2002) also found this to be the case when recording a single released individual in Mexico using a time-expansion system.

Spix's disc-winged bat (*Thryoptera tricolor*) is the only representative of the family Thyropteridae in northern Central America. Three individuals were released under controlled conditions and passive monitoring stations were set adjacent to known roosts on 3 nights, without any calls being detected. This species also appears to be too quiet to detect under field survey conditions. Others have recorded calls of this species under controlled conditions and found that calls were of low intensity (Fenton et al. 1999). Based on these results, it not practical at this time to include these species for consideration during acoustic surveys.

Many Neotropical Vespertilionids are readily identified by echolocation calls (e.g. *Eptesicus* spp., *Lasiurus* spp., *Rhogeessa tumida*). However, this family contains problematical species, particularly in the genus *Myotis.* This genus appears to present identification problems worldwide regardless of the method of detection used. In addition, there is the general difficulty of separating very closely related species, which stems in many cases from problems of identifying live bats in the hand. Without the benefit of skulls, many species are difficult to identify with confidence. The danger has been that acoustic "vouchers" or reference files may have been attributed to a species of *Myotis* that was not correctly identified in the hand, particularly in the U.S. where taking of voucher specimens is restricted. This may cause confusion when the call sequences do not match those of other individuals reputed to be the same species. The possibility exists that some of these errors in identification have been interpreted as "variation" within a given species, when in fact they may have been recorded from distinct species to begin with.

Some species of Molossidae are also problematic. These rapid and high-flying species are often difficult to capture for confirmation except at water sources or known roost sites. Recordings at roosts may not be diagnostic, since calls given when bats leave roosts are generally not characteristic of search phase calls but similar to a "high clutter" call. Changes in nomenclature and what constitutes a recognized taxa confound understanding of geographic ranges without the ability to physically match known individuals to calls. Further, some authors have suggested "plasticity" in search-phase calls for species such as *Molossus molossus* (e.g., Kössl et al. 1999), but this variation may simply be calls that differ from standard search-phase calls (e.g., leaving roosts, commuting calls, etc.).

While not every species currently can be identified by echolocation calls alone, a large number can. With many shared species throughout the Neotropics, the compilation of an acoustic library of confirmed calls for species is making it easier for those wishing to identify free-flying bats based on echolocation calls. It is critical that before embarking on such projects in the Neotropics, researchers have a range of calls based on confirmed species identifications to use as a comparative reference. To this end, the Neotropical Bat Information Database (NEOBAT; *http://fwie.fw.vt.edu/wcs/*) was created. Echolocation calls are displayed for each verified species providing both the output from the Anabat system and spectral analysis recorded using the Pettersson D980 in time-expansion mode. This provides a means for comparing field recordings with confirmed voucher records.

How effective are acoustic surveys in the Neotropics? For the majority of species, they appear to be extremely effective. If call sequences are of sufficient length to view the range of normal variation, then identification is not difficult. Care must be taken when working in areas where complete call libraries have not yet been complied. Currently, headway is being made with methods to identify Molossids and species of *Myotis*. Many of these species have yet to be recorded. For identifying Neotropical species, more detail is better – not more details from a limited range of calls, but more call sequences to fully understand the range of variation within a given species.

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APPENDIX: Bat species in Belize that can be identified by echolocation call sequences.

Emballonuridae (sac-wing bats)	**Vespertilionidae (evening bats)**	**Mormoopidae (leaf-chinned and mustached bats)**	**Molossidae (free-tailed bats)**
Balantiopteryx io	*Bauerus dubiaquercus*	*Mormoops megalophylla*	*E. underwoodi*
Centronycteris centralis	*Eptesicus furinalis*	*Pteronotus davyi*	*Molossops greenhalli*
Diclidurus albus	*Lasiurus blossevillii*	*Pteronotus gymnonotus*	*Molossus molossus*
Peropteryx kappleri	*L. ega*	*Pteronotus parnellii*	*M. rufus*
Peropteryx macrotis	*L. intermedius*	*Pteronotus personatus*	*M. sinaloae*
Rhynchonycteris naso	*Myotis elegans*		*Nyctinomops laticaudatus*
Saccopteryx bilineata	*M. keaysi*	**Noctilionidae**	
Saccopteryx leptura	*Rhogeessa tumida*	**(bulldog or fishing bats)**	
		Noctillio leporinus	

NEOTROPICAL LEAF-NOSED BATS (PHYLLOSTOMIDAE): 'WHISPERING' BATS AS CANDIDATES FOR ACOUSTIC SURVEYS?

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The speciose Neotropical leaf-nosed bats (Phyllostomidae) fulfill a wide range of ecological functions (pollination, seed dispersal, predation) and are therefore of special interest for conservation efforts. In contrast to aerial insectivorous bats whose echolocation calls are being increasingly used for species identification in inventories and monitoring programs, knowledge about echolocation by phyllostomid bats is still poor. This is due to difficulties in recording the low-intensity and high-frequency signals of this group, and in classifying the comparatively uniform call structure. Typically, echolocation signals of leaf-nosed bats consist of short (< 2 ms), multiharmonic, and steeply frequency-modulated (FM) downward sweeps. This call type is seen as an adaptation to obstacle-filled, cluttered habitats, such as the forests where most phyllostomid bats glean food from surfaces. Ongoing field and laboratory studies reveal that call structure in leaf-nosed bats is more variable within and across species than previously thought. For instance, when flying in open space above the canopy, the greater spear-nosed bat, *Phyllostomus hastatus*, emits long (> 5 ms), low-frequency echolocation calls that are well suited to acoustic identification and monitoring. Furthermore, it is likely that for many phyllostomid bats, an assessment of best or peak frequency of their search calls, number of harmonics, sweep rate, and pulse interval will allow classification according to body size and in some cases, also to species. In-depth studies of the largely unknown degree of inter- and intra-specific variation in call design are needed to advance our knowledge of call design in phyllostomid bats to allow acoustic identification.

Key words: acoustic monitoring, call structure, echolocation, Phyllostomidae, species identification

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INTRODUCTION

Tropical forests harbor the highest number of bats (Chiroptera) worldwide. For instance, in Neotropical lowland forests, 80-100 species are known or expected to coexist (e.g., Kalko 1998; Kalko et al. 1996; Simmons and Voss 1998). The endemic family of New World leaf-nosed bats (Phyllostomidae) with more than 140 species (Koopman 1993) has the highest ecological diversity. In this family, diets range from fruit, pollen, nectar, and leaves to insects, small vertebrates, and blood (e.g., Findley 1993). Because of their high biomass, abundance, mobility, and dietary diversity, phyllostomid bats play key roles in important ecological processes such as pollination, seed dispersal, and predation (e.g., Findley 1993; Kalko 1998). However, human influence, through changes in land use and exploitation of natural resources, are altering habitat types and landscapes at an ever-increasing pace. These anthropogenic disturbances lead to fundamental changes in the composition, and presumably the function, of bat communities at local and regional scales (e.g., Brosset et al. 1996; Fenton et al. 1992). Consequently, establishment of conservation-oriented management programs is crucial to maintain diversity and ecological functions of key taxa such as bats. To achieve this goal, comprehensive inventories are necessary, as are methods that allow researchers to conduct inventories quickly, reliably, and cost-effectively. Ideally, these methods should also offer a reasonable prospect for long-term monitoring protocols.

Currently, mist netting represents the method of choice for inventories and surveys of leaf-nosed bats (e.g., Fenton et al. 1992; Findley 1993; Kalko 1998; Simmons and Voss 1998). Although it is an effective tool, mist netting is time- and labor-intensive and has the disadvantage of creating net-shyness if the intervals between netting sessions are too short. Furthermore, mist netting is frequently limited to small areas, and it samples groups of bats differently. Aerial insectivores are notoriously difficult to catch with mist nets and therefore often go undetected in mist-netting studies. Thus, species identification of these bats based on the shape and pattern of their echolocation calls has become a successful and time-conserving method (for summaries see this volume and Gannon and Bogdanowicz 2000).

In addition to species identification, acoustic monitoring also allows the documentation of presence/absence of species, habitat use, and activity patterns. This approach has been used successfully in the temperate zone and is becoming more common in the Neotropics (e.g., Kalko et al. 1996; O'Farrell and Miller 1997, 1999; Miller this volume).

In contrast to Neotropical aerial insectivorous bats (Emballonuridae, Mormoopidae, Noctilionidae, and Vespertilionidae), echolocation behavior of the speciose Phyllostomidae has been little studied to date (e.g., Barclay et al. 1981; Belwood 1988; Howell 1974; Kalko and Condon 1998; Thies et al. 1998). The main reason for this lack of knowledge is the difficulty in recording the low-intensity, high-frequency calls of most leaf-nosed bats which are often classified as "whispering bats" (e.g., Fenton 1995; Schnitzler and Kalko 1998, 2001). Although leaf-nosed bats occupy a wide range of trophic levels, call structure appears rather stereotyped. However, ongoing field and laboratory studies suggest that call structure of leaf-nosed bats is more variable within and across species than previously thought. Given the successful use of acoustic identification and monitoring for aerial insectivorous bats, this raises the question about the degree to which phyllostomid bats can be assessed and identified acoustically in the field.

Here, I summarize the current knowledge of echolocation behavior by leaf-nosed bats, with special emphasis on possibilities for species identification and applicability of acoustic surveys. I address inter- and intraspecific signal variability, as well as differences in emission patterns, and link them to habitat type (open versus cluttered space), where the bats are foraging, and body size (large versus small). I identify which phyllostomid bats might be well suited for acoustic species identification and monitoring based on the detectability of echolocation calls with bat detectors and on diagnostic signal characteristics. Finally, I propose future directions to enlarge the existing database and suggest how to conduct acoustic surveys for leaf-nosed bats in field studies.

METHODS

The data presented here were generated during studies conducted between 1993 and 2001 on Barro Colorado Island, a field station of the Smithsonian Tropical Research Institute in Panama. Barro Colorado Island, a 15.6 km^2 island near the Panama Canal in Lake Gatun, is covered with semi-deciduous, moist tropical lowland forest of different successional stages ranging from younger forest (80-100 years) to tall, old-growth forest (400-600 years) (Leigh 1999). A pronounced dry season from the end of December until the middle of April with almost no precipitation is followed by an extended rainy season where more than 90% of the annual rainfall (about 2600 mm) occurs.

All echolocation recordings were made from identified phyllostomid bats in flight. To simplify call description, signals that were emitted by phyllostomid bats without an obvious reaction towards obstacles, food, or conspecifics as indicated by a distinct shortening of pulse duration and pulse interval are classified as search calls (Schnitzler and Kalko 1998). With the exception of *Phyllostomus hastatus*, *Artibeus jamaicensis*, and *Micronycteris microtis*, the bats were hand-released after they had been captured in mist nets set along trails in the forest, identified, and measured. *Phyllostomus hastatus* flying in open space was identified based on its characteristic size and flight style. Free-flying *Artibeus jamaicensis* were recorded in the field while foraging at a fig tree. Mist-net captures and recordings of bats in a flight cage confirmed the species identifications. Echolocation signals of *M. microtis* were documented while individuals were foraging in a flight cage (approximately 4 m long x 4 m wide x 2.5 m high) on Barro Colorado Island. To maximize the quality of sound recordings of hand-released bats, an ultrasound microphone was placed about 1 m in front of the bat. Due to the directionality and low intensity of sound emission in phyllostomids, the position of the microphone was adjusted as soon as the bat took flight to keep it on-axis as much as possible. This also applied for the field recordings of free-flying *P. hastatus* and *A. jamaicensis*.

The frequency response of the custom-made condenser microphone (University of Tuebingen, Germany) was ±3 dB between 30 and 120 kHz with a drop of 0.2 dB/kHz below 15 kHz and up to 200 kHz. The signals were amplified, digitized (sampling rate 312.5 kHz), stored in a buffer (3.3 s real time), and then read out at 1⁄15 the original speed. After digital to analog conversion, the time-expanded sequences were recorded onto a WM-DC6 Sony Walkman using 90-min chrome-dioxide cassettes. The slowed-down calls were made audible in the field by a built-in, real-time heterodyning system while the time-expanded signals were recorded onto the tape recorder. The time-expanded signals were analysed with Avisoft-SASLabPro©. The calls were digitized (sampling rate 24 kHz) and processed through a Fast Fourier Transformation (FFT) using a Hanning Window. For individual calls, an FFT with 256 points and a time overlap of 87.5% was chosen. For search sequences, an FFT with 512 points and a time overlap of 75% was applied. For this paper, the signals are presented as spectrograms and oscillograms (amplitude) without filtering to illustrate the relation between echolocation-signal intensity and the natural background noise. In the spectrogram, intensity is encoded in shades of grey: black codes for high intensity, white for low intensity. To allow direct visual comparison of the spectrograms, individual calls are depicted in 10-ms steps and call sequences in 1500-ms blocks, both with standardized frequency axes.

The echolocation data I present are limited to a qualitative assessment of call structure and variability in phyllostomid bats. The morphometric measurements of the bats originate from a minimum of 10 animals per species that were caught on Barro Colorado Island (in litt.). They are given as mean (body mass in g) and as range (forearm [FA] length in mm).

RESULTS

All phyllostomid bats studied echolocated continuously in flight. Most search calls consisted of short, multi-harmonic, high frequency, low-intensity, and steep frequency-modulated (FM) downward sweeps (Figs. 1-7 and 8b). Some harmonics were incomplete because of signal filtering through the vocal tract (for further details see Thies et al. 1998). The main energy was concentrated in two harmonics (Figs. 2-4). In sound recordings with very good signal-to-noise ratios, more harmonics (up to 5) were visible (Figs. 5a, 5b, 8b and 9c).

Comparison of search calls from fruit-eating bats suggests that lower harmonics are more pronounced in larger bats than in smaller ones, where higher harmonics are more prominent. For example, most signal energy in the search calls of the large fruit-eating bat *Artibeus lituratus* (61 g; FA 69-78 mm) resided in the 2nd and 3rd harmonic (Fig. 1) in contrast to the small *Artibeus watsoni* (11 g; FA 35-42 mm), where most call energy was concentrated in the 3rd and 4th harmonic (Fig. 2). This result is also consistent with a previous study on the echolocation behavior of 2 different-sized species of *Carollia* on Barro Colorado Island where, in the larger species, *C. perspicillata* (19 g; FA 41-45 mm), the second and third harmonic contained most call energy in contrast to the smaller species, *C. castanea* (13 g; FA 34-38 mm) with most call energy in the 3rd and 4th harmonic (Thies et al. 1998).

This pattern needs to be interpreted with caution, as the comparison of 2 medium-sized fruit-eating bats, *Carollia perspicillata* (19 g; FA 41-45 mm) and *Chiroderma villosum* (22 g; FA 42-47 mm) shows (Figs. 3 and 4). Although both bats feed on fruit, forage mostly inside the forest, and are similar in size, the search signals differ in the composition of the harmonics and in the distribution of energy. The call energy in *C. villosum* was concentrated at much higher frequencies than in *C. perspicillata*. Furthermore, the call energy in *C. perspicillata* was not only at a much lower frequency (Fig. 3) than in the similar-sized *C. villosum* (Fig. 4), but it was also lower than the frequency of the much larger *A. lituratus* (Fig. 1).

The reasons for this discrepancy are likely to include the inherent difficulties in recording the low-intensity calls of phyllostomid bats and a high intraspecific variability in call structure. The design of the echolocation calls depends to a large degree on the behavioral situation of the bats emitting the signals. In particular, the distance of bats to clutter-producing objects and whether they are searching for food or approaching specific objects such as obstacles or roosts strongly influence signal design (e.g., Fenton 1995; Kalko and Schnitzler 1998; Schnitzler and Kalko 1998, 2001). For instance, when flying in open space, the common fruit bat *A. jamaicensis* produced relatively long calls (3-5 ms) with signal energy concentrated in the lower parts of the 2nd and 3rd harmonic (Fig. 5a). These calls were also relatively loud and could be picked up with a bat detector at distances of 5-10 m. The call represented here (Fig. 5a) originates from an *A. jamaicensis* foraging at a fig tree close to the shoreline of Barro Colorado Island as the bat flew from the fig tree into the open space above Lake Gatun. As soon as *A. jamaicensis* flew from the open towards the fig tree with ripe figs, pulse structure changed in a predictable way. Sound duration and pulse interval were shortened, calls were emitted in groups of several signals, and the energy content of the signal was moved into the higher frequency range of the harmonics (Fig. 5b). The shortest sound duration and highest frequencies occurred when *A. jamaicensis* approached a ripe fig (Fig. 5c), grasped it in flight, and carried it to a temporary feeding roost.

Echolocation calls of flying frugivorous phyllostomid bats (Figs. 6, 7 and 8b) are remarkably similar to the search calls emitted by gleaning insectivorous leaf-nosed bats (Figs. 1-5), with similar trends in terms of body size, composition of harmonics, and concentration of signal energy. For example, the medium-sized, insectivorous d'Orbigny's round-eared bat, *Lophostoma silvicolum* (34 g; FA 50-56 mm), produced search calls with the energy concentrated in the 2nd and 3rd harmonic (Fig. 6). As expected, call energy of the smaller, insectivorous little big-eared bat, *Micronycteris microtis* (7 g; FA 32-37 mm), was also in the 3rd and probably the 4th harmonic (Fig. 7). Because the search calls of *M. microtis* were so low in intensity and high in frequency, only 1 harmonic was typically recorded. However, some recordings revealed that *M. microtis* also produces multi-harmonic signals with up to 4 harmonics present.

In general, the higher harmonics of echolocating leaf-nosed bats are frequently lost due to a low signal-to-noise ratio and the lower sensitivity of most microphones in the high-frequency range (> 50 kHz), even when a bat is on-axis within 1 m of the microphone. The loud and often high-frequency background noise produced mainly by insects further complicates detection and identification of phyllostomid calls (Fig 7, 9a).

In contrast to the previous examples, the large, omnivorous greater spear-nosed bat, *Phyllostomus hastatus* (126 g; FA 80-93 mm), flying in open space above canopy or ground, is characterized by loud, long (> 5 ms), and relatively low-frequency (most call energy < 50 kHz) echolocation calls (Fig. 8a). These calls could be detected over long distances (> 10 m) with bat detectors. As for all phyllostomid bats studied so far, the search signals were composed of several harmonics. There is a characteristic pattern in the distribution of the call energy within the signals. The energy was at first concentrated in the lower (2nd) harmonic and was subsequently shifted after about one third of the call into the higher (3rd) harmonic. Search calls emitted by *P. hastatus* flying close to vegetation (Fig. 8b) or approaching targets (see Kalko and Condon 1998) differed substan-

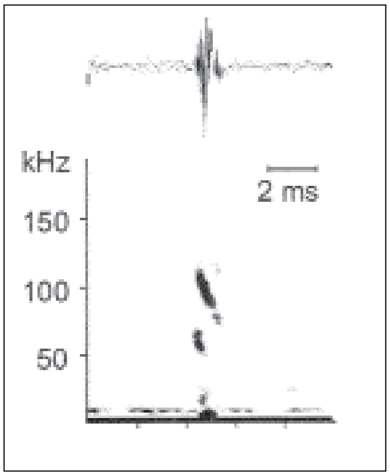

Figure 1: Search call of the frugivorous bat *Artibeus lituratus* in flight.

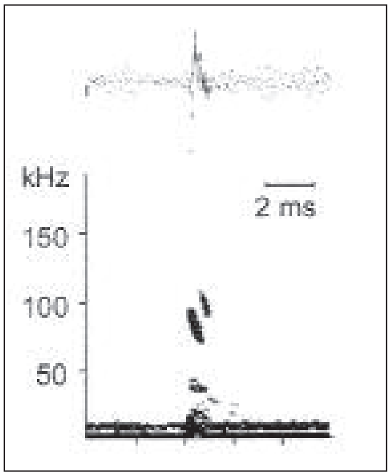

Figure 2: Search call of the frugivorous bat *Artibeus watsoni* in flight.

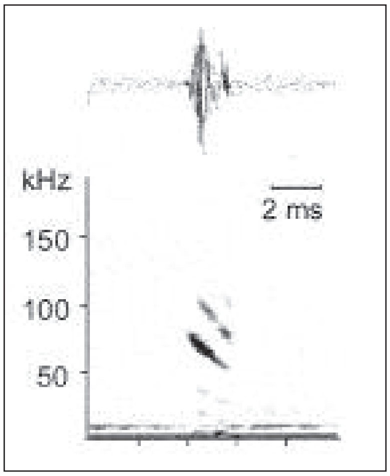

Figure 3: Search call of the frugivorous bat *Carollia perspicillata* in flight.

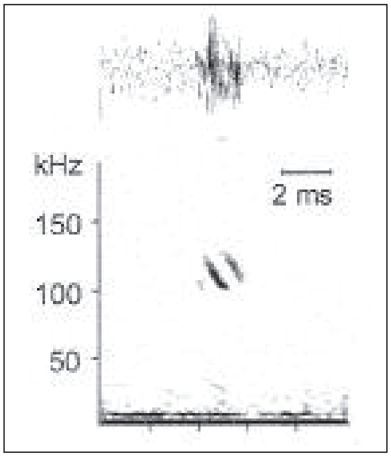

Figure 4: Search call of the frugivorous bat *Chiroderma villosum* in flight.

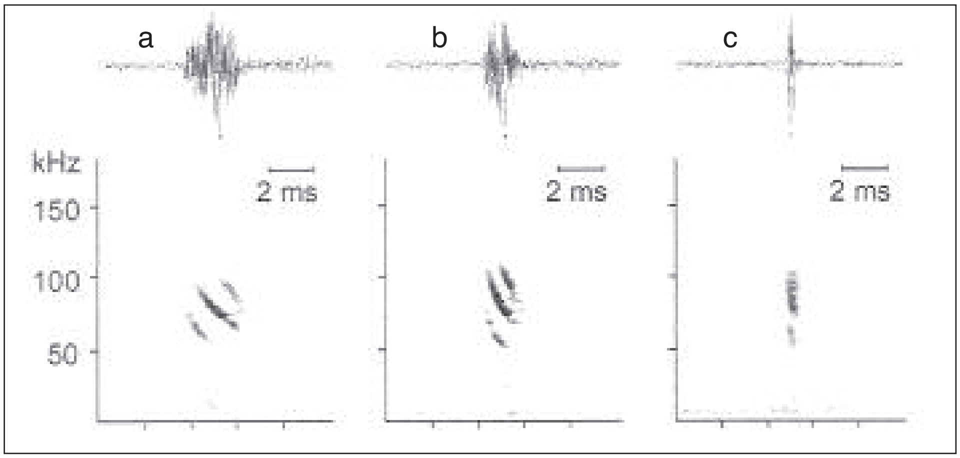

Figure 5: Search call of the frugivorous bat *Artibeus jamaicensis* flying in **a:** open space, **b:** close to clutter (vegetation), and **c:** approaching a ripe fig.

tially from calls emitted in the open. The calls were much shorter and did not exhibit the distinct energy shift between the 2nd and 3rd harmonic.

Pulse interval (measured from the start of one call to the start of the next) also differs among phyllostomid bats. Similar to the examples above, these differences are likely linked in part to body size of the bats and to the behavioral context in which the recordings were made. For example, when flying close to clutter, the medium-sized *L. silvicolum* emitted calls in a regular pattern with pulse intervals ranging between 20-40 ms (Fig. 9a). This corresponds to about 2 to 4 calls per wing beat. In contrast, search sequences of the larger *A. jamaicensis* flying away from a fig tree into open space used longer pulse intervals (60-80 ms), which likely correspond to 1 or 2 search signals per wing beat (Fig. 9b). The longest pulse intervals (80-120 ms) were recorded from *P. hastatus* when it flew in open space above the canopy (Fig. 9c). All data are for search sequences when the bats were commuting or searching for food without a reaction to a specific target. As has been shown for *C. perspicillata*, *C. castanea*, and *P. hastatus*, structure and pattern of call emission change drastically when bats approach targets (see Kalko and Condon 1998; Thies et al. 1998). In particular, calls are not emitted singularly but in groups of 3-4 or more, correlated with wing beat.

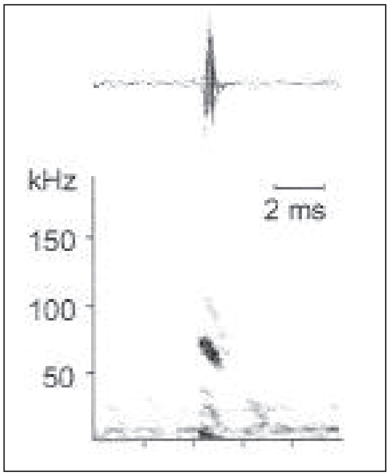

Figure 6: Search call of the insectivorous bat *Lophostoma silvicolum* in flight.

DISCUSSION AND CONCLUSIONS

These results confirm those of previous studies and experiences indicating that echolocation calls of most phyllostomid bats are difficult to record because of their high frequency and low intensity. Often, successful recordings are only possible when the bats are on-axis within 1-3 m of the microphone. This makes recording echolocation calls of most leaf-nosed bats for inventories and monitoring purposes impractical. Furthermore, the uniformity of calls limits detailed acoustic species identification of leaf-nosed bats to a few species.

What are the underlying reasons for the uniformity in signal design? The short, steep FM calls of phyllostomid bats are seen as an adaptation to obstacle-filled, echo-cluttered environments such as the forests where most leaf-nosed bats glean food from surfaces (Kalko and Schnitzler 1998; Schnitzler and Kalko 1998, 2001; Simmons and Stein 1980). Short, steep, and low-frequency FM calls are well suited for orientation in cluttered space but are less suited to finding non-moving food, such as fruits or insects sitting on leaves. Because of the difficulty of distinguishing echoes reflecting off targets (food) from those reflecting from obstacles (branches, leaves), especially when these echoes overlap, phyllostomid bats often use a combination of sensory cues (echolocation, olfaction, vision, prey generated sounds) to detect prey (e.g., Kalko and Schnitzler 1998, 2001).

The similarity in sensory tasks that leaf-nosed bats perform is reflected in the high degree of convergence in signal structure among frugivorous, insectivorous, nectarivorous, sanguivorous, and carnivorous species. In contrast, leaf-nosed bats that do not glean food from surfaces or do not fly in cluttered habitats exhibit striking differences in call design, as is the case for *P. hastatus* flying in the open. It produces louder and longer echolocation calls than any other phyllostomid species studied to date. The characteristically long echolocation calls of *P. hastatus* can be picked up with a bat detector at distances of 5-10 m or more. This makes *P. hastatus* a good candidate for acoustic surveys. However, the long and loud calls are only emitted when *P. hastatus* flies in the open.

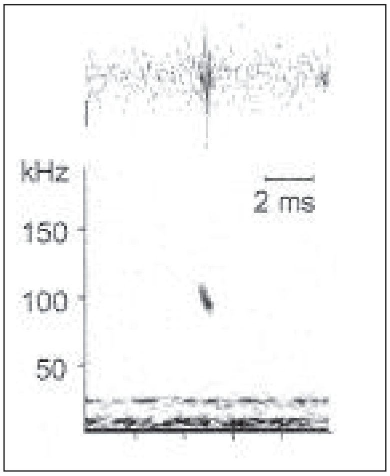

Figure 7: Search call of the insectivorous bat *Micronycteris microtis* in flight.

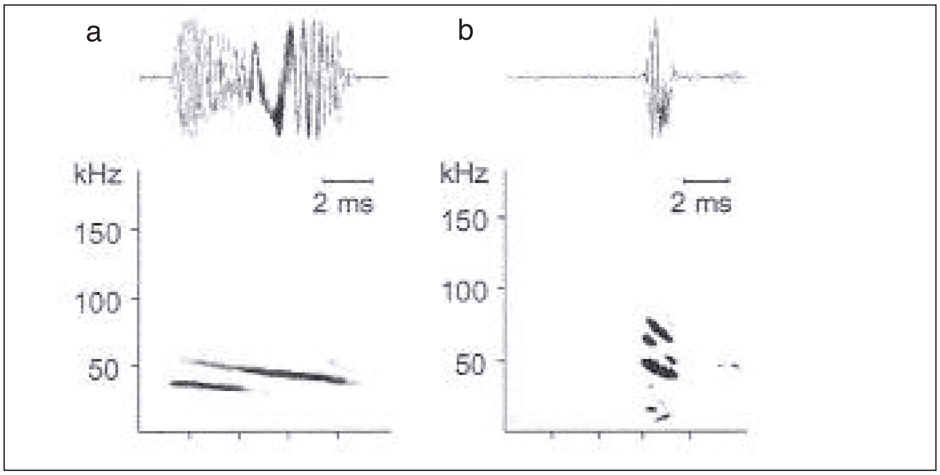

Figure 8: Search call of the omnivorous *Phyllostomus hastatus* flying in **a:** open space above the canopy, and **b:** close to clutter (vegetation).

Despite the uniformity of call design in phyllostomid bats, certain trends occur, which, if confirmed, could improve the use of acoustic information in this group. For instance, as in aerial insectivorous bats (see summary in Jones 1999; Schnitzler and Kalko 1998), there is a trend for higher frequencies in smaller leaf-nosed bats than in larger species (e.g., Thies et al. 1998). Furthermore, there is a trend towards longer pulse intervals with increasing body size. In addition to body size, habitat parameters such as the distance to clutter also play important roles in shaping pulse design and pattern of emissions. As for aerial insectivores, leaf-nosed bats flying in the open emit longer calls at longer inter-pulse intervals than in more closed habitats (e.g., Jones 1999; Schnitzler and Kalko 1998). This relationship holds true across a variety of feeding guilds including frugivorous and insectivorous bats.

There are currently only a few data from a few species available and because of this, most results are limited to preliminary, qualitative assessments. Thus the true extent of intra- and interspecific variability in call design and pulse pattern has yet to be determined. We need quantitative, in-depth studies of a range of different-sized species with different ecologies. It is likely that for many phyllostomid bats, assessment of best or peak frequency of search calls, number of harmonics, sweep rate, and pulse interval will allow further classification. The results of those studies can then be integrated into modern analysis tools such as discriminant function analysis or multivariate statistics and neural networks to search for characteristic, species-specific patterns (e.g., Jones and Parsons, this volume; Parsons 2001) and probably also to link strongest frequency with body size.

The prerequisites for quality recordings of phyllostomid echolocation calls are highly sensitive microphones with an excellent signal-to-noise ratio and a good frequency response in the higher-frequency range (> 50 kHz). Good-quality, on-axis sound recordings reduce the ambiguities inherent in incomplete recordings. Low signal-to-noise ratios and limited frequency range quickly lead to the loss of harmonics and of other characteristic call parameters, such as the energy distribution within a call. Furthermore, because of the importance of call structure for species identification, recording and analysis of phyllostomid echolocation calls require the preservation of harmonics.

With an extended database, it is likely that acoustic monitoring of leaf-nosed bats will contribute more to acoustic surveys and monitoring programs. Already it is clear that some species such as *P. hastatus* are well suited for acoustic surveys because of their loud, low-frequency calls. Phyllostomid echolocation could also be used for acoustic surveillance of nectarivorous and frugivorous bats visiting flowers and fruits. By placing microphones close to potential food items, the frequency and duration of the visits and probably also the size classes of the visiting bats could be measured.

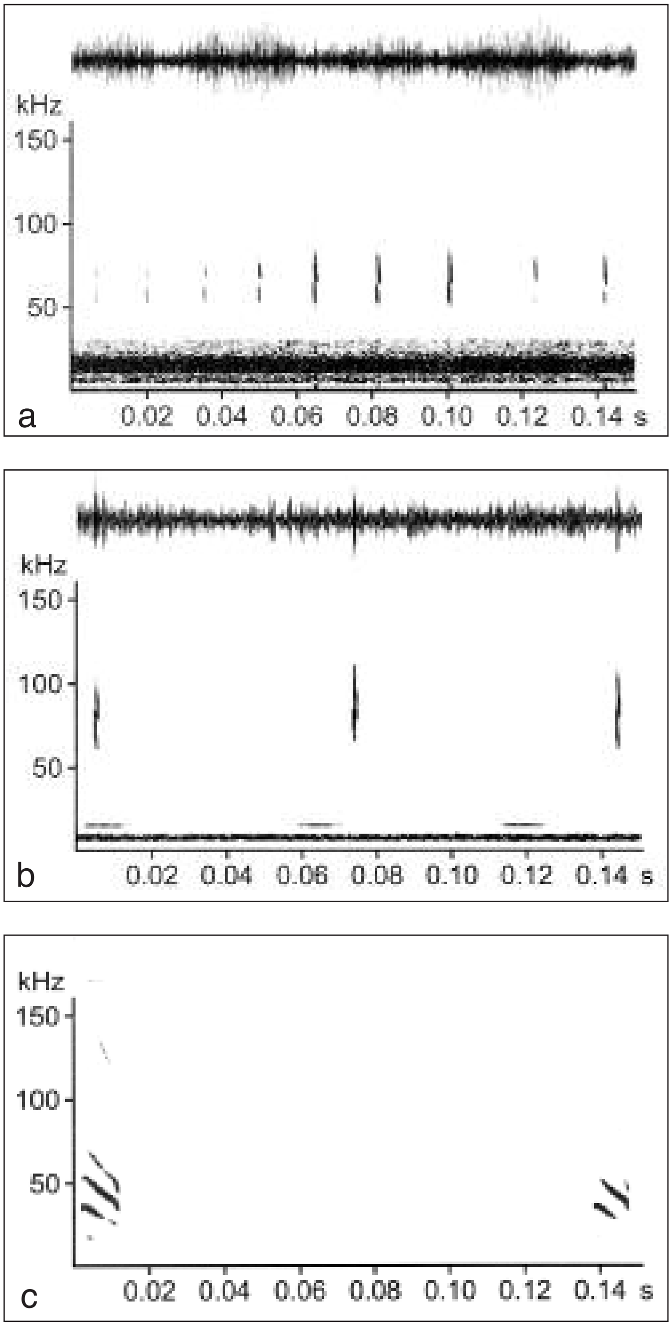

Figure 9: Search sequence of **a:** *Lophostoma silvicolum* flying close to clutter (vegetation), **b:** *Artibeus jamaicensis* flying in open space near a fig tree, and **c:** *Phyllostomus hastatus* flying in open space above the canopy.

ACKNOWLEDGMENTS

I thank the Smithsonian Tropical Research Institute in Panama for excellent working conditions and the many bat people who have joined me in my endeavors over the years. In particular, I wish to thank Hans-Ulrich Schnitzler for his continuous support, Charles Handley for introducing me to the fascinating world of Neotropical bats, and Marco Tschapka for his valuable comments on the manuscript.

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Section 3

ULTRASOUND SPECIES IDENTIFICATION

FIELD AND LABORATORY APPLICATIONS

HETERODYNE AND TIME-EXPANSION METHODS FOR IDENTIFICATION OF BATS IN THE FIELD AND THROUGH SOUND ANALYSIS

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In Europe, ultrasound detectors are used to conduct various types of fieldwork on bats, including ecological research, area surveys, and monitoring of populations. The heterodyne system has been used extensively during the last 25 years and is still the most common. To enable the recording of information about frequencies, the frequency-division system was commonly used in Europe, singly or in combination with heterodyne. In 1985, time expansion became available, and this system is now widely used in combination with the heterodyne system for identifying bat species in the field. In this paper, I assess the utility and limitations of the heterodyne and time-expansion systems using examples from work on the European bat fauna. The advantage of the two systems in combination is improved detection of bats, instant identification of species, and the ability to subsequently analyze recordings. Further, I illustrate the importance of sampling species-specific sequences and being aware of various limitations and pitfalls. Even the most skilled observers need to accept that it is not always possible to identify species. Separating similar species often requires long periods of observation during which bats can be heard (and seen) hunting or performing characteristic behaviors. Therefore, in many studies, some species must be pooled into groups, e.g., using line transects when there is limited time for each observation.

Key words: bat detectors, Chiroptera, European bat species, field identification, frequency division, heterodyne, monitoring, surveys, time expansion, ultrasound

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INTRODUCTION

Beginning in the late 1970s, ultrasound detectors have been used for identification and field studies of bats in Europe with new techniques being continuously developed (e.g., Ahlén 1981, 1990; Ahlén and Baagøe 1999; Ahlén and Pettersson 1985; Andersen and Miller 1977; Limpens and Roschen 1995; Miller and Degn 1981; Weid 1988; Weid and von Helversen 1987; Zingg 1990) Technological improvements and experience with different systems have led to an expanding knowledge of different species, including how they can be identified and observed in nature. The purpose of this paper is to describe two ultrasound-conversion systems, heterodyne and time expansion, and evaluate the advantages and limitations of each system.

FEATURES OF THE HETERODYNE SYSTEM

The heterodyne system is sensitive and enables long-range detection of bats. Therefore, it is perhaps the best system to detect the most bats. The narrow frequency band that is transposed to audible sound must be tuned to the sounds made by the bat. This means that there is a risk of missing a bat even at short range. Careful tuning will allow an observer to determine whether there is a constant-frequency or near-constant-frequency component (tonal quality) in the sound and at approximately which frequency. FM sweeps can be described as dry clicks, while quasi-constant frequency components sound like smacking or drops of water, and longer constant-frequency components sound tonal or whistling. A number of other sound qualities can be heard using a heterodyne system but all are difficult or impossible to measure. Using most heterodyne detectors it is impossible to save frequency information other than remembering the tuning. Therefore, a combination of heterodyne and frequency-division system (Andersen and Miller 1977) represent a solution to that problem, and were commonly used until time expansion became available.

The heterodyne system enables sampling of long sequences of pulses which, when displayed as oscillograms, allow analyses of pulse repetition or rhythm.

FEATURES OF THE TIME-EXPANSION SYSTEM

The time-expansion system preserves the unchanged original sound with the high frequencies and harmonics. It can be played back at a slower speed, typically 10 times slower (20 times for very high frequencies). In principle this makes the whole spectrum of bat sounds audible, and it is simple to save by using a relatively inexpensive recorder. For the best results, one has to learn how to choose the right moment to trigger the system; thereby saving the best signals and avoiding over-

Groups	Genera	No. of species
CF *Rhinolophus*		5
FM *Myotis*		10
Plecotus	4	
QCF *Nyctalus*	4	
Eptesicus	3	
Vespertilio		1
Pipistrellus		5
Tadarida	1	
Miniopterus		1
Barbastella		1

Table 1: Number of species in Europe belonging to the sonar groups CF, FM and QCF.

load. All information on frequencies and relative amplitude is preserved, as well as the shape and other features of the single sound pulses. This is an excellent system for recording short samples of sounds to be analyzed afterwards. This advantage was recognized immediately when the system became available. However, even to well-trained observers, the value of using time expansion for species identification in the field has only been slowly recognized.

Sonar Characteristics of European bats

The 35 bat species found in Europe use many different types of vocalizations for sonar and social communication. As far as we know, no two species use identical vocalizations, but species can be grouped according to the general features of sound. One way to group them is to use the sonar components that are most useful for species identification, namely CF (constant frequency), FM (frequency modulation), and QCF (quasi constant frequency, *quasi = L. as if, almost*; Table 1).

The grouping perhaps represents an oversimplification, because members of all three groups use FM sweeps and even true-CF bats occur in the third group. The names represent the most common components used for orientation. Social calls are variable in structure and are much more complicated to describe or classify, even though they are of great use for identifying species. Figs. 1-6 provide examples of sonar sounds produced by members of the three groups.

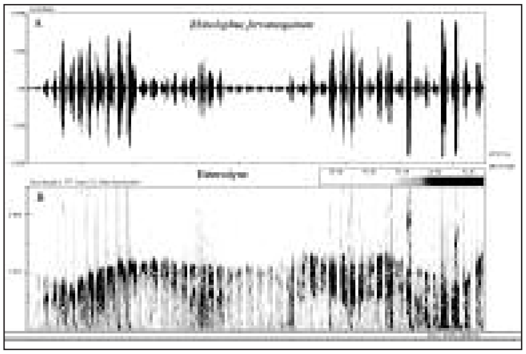

Figure 1: Heterodyne representation of CF sonar call from *Rhinolophus ferrumequinum*. Upper diagram **A** is an oscillogram, lower diagram **B** is a sound spectrogram, Note that frequency varies due to Doppler shifts when the bat is flying.

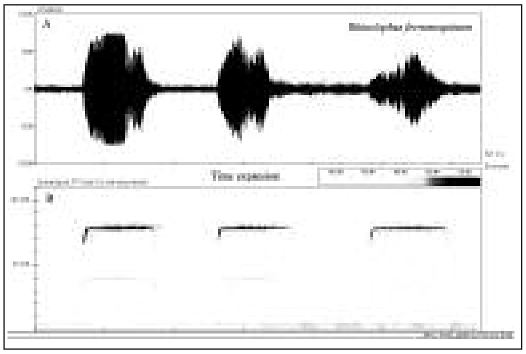

Figure 2: Time-expansion representation of CF sonar call from *Rhinolophus ferrumequinum*. Upper diagram **A** is an oscillogram, lower diagram **B** is a sound spectrogram (sonogram).

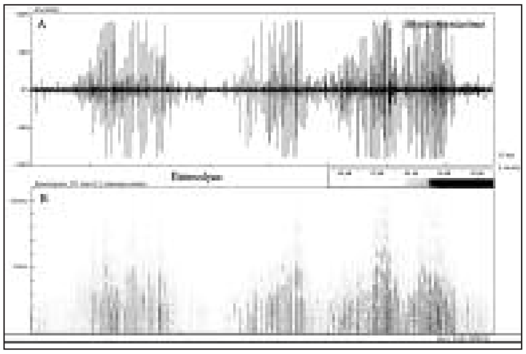

Figure 3: Heterodyne representation of FM sonar from *Myotis mystacinus* (**A** = oscillogram, **B** = sound spectrogram).

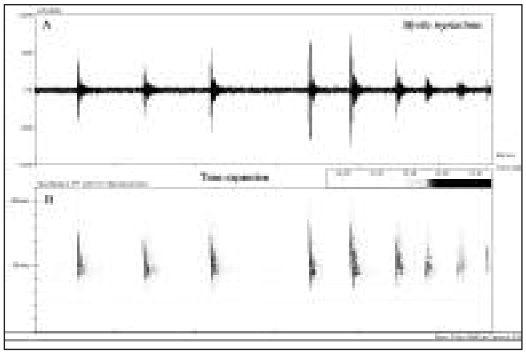

Figure 4: Time-expansion representation of FM sonar from *Myotis mystacinus* (**A** = oscillogram, **B** = sound spectrogram).

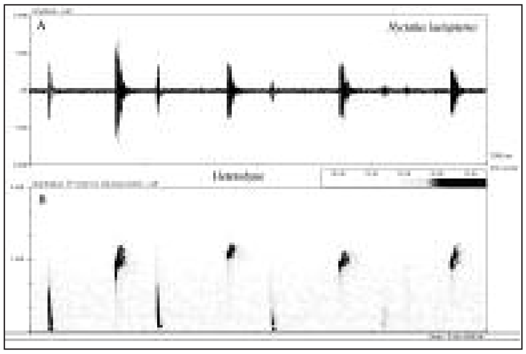

Figure 5: Heterodyne representaton of QCF sonar from *Nyctalus lasiopterus* (**A** = oscillogram, **B** = sound spectrogram).

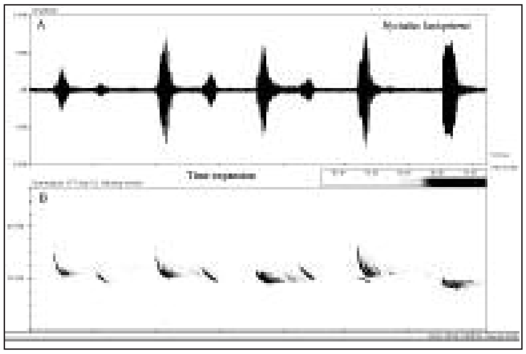

Figure 6: Time-expansion representaton of QCF sonar from *Nyctalus lasiopterus* (**A** = oscillogram, **B** = sound spectrogram).

QUANTIFYING SOUND FEATURES

To quantify characteristics of sounds heard directly through detectors or from recordings, it is necessary to make measurements. The following remarks are important in the context of taking measurements to identify species.

With a heterodyne detector, one can only turn a knob and read a scale or display to assess frequency. The values are not saved. This means that it is difficult to check afterwards if the tuning was correctly made. Also, some very common bat sounds with near-CF endings (QCF-type), may have a broad band of frequencies (5 kHz or more) where the heterodyned signal sounds exactly the same. Indeed, some observers who use heterodyne argue about bats being 1 or 2 kHz too low or high! Added to this uncertainty is the Doppler effect that may change the frequency by 1 or 2 kHz.

With a time-expansion system, the CF and near-CF-frequencies can be heard and perceived immediately after recording. Workers with absolute pitch abilities can differentiate frequencies within a few kHz, that is, with better precision than with heterodyne tuning. With a sound-analysis program, the sounds can be inspected and measured. Even with the best recordings and diagrams, however, there are some uncertainties that must be kept in mind when characterizing species and making comparisons.

Some uncertainties are due to changes in sound that occur as calls travel from the bat to the detector, or changes produced by the instruments themselves. Pulse length or maximum frequency is often variable because the first low-intensity part of the pulse can easily be lost in the recording. These measurements are seldom of diagnostic value. The frequency at maximum amplitude, the power spectrum peak (whole or part of pulse), frequency at the QCF-ending, and the so-called best-listening frequency (using heterodyne) are examples of repeatable measurements. Biologically, the most significant frequency in such pulses is perhaps the part that creates the most powerful echoes, which are easily seen in some sonograms. This frequency is usually the last strong part of the QCF-ending and typically corresponds to the best-listening frequency in heterodyne tuning where the sound is most drop-like. This frequency typically coincides with the frequency at maximum amplitude (Fig. 7). The power spectrum peaks look exact, but this can be misleading because both amplitude and duration create the spectrum.

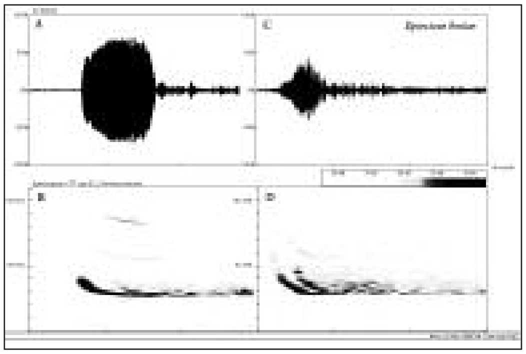

Figure 7: Two single pulses of the same pulse train from a passing *Eptesicus bottae*. The difference illustrates pulse-to-pulse variation, with the dominant echo-producing frequency being constant (**A**, **C** = oscillograms, **B**, **D** = sound spectrograms).

For obvious reasons, the different means to measure pulse features often cause confusion when the methods are not specified. It is, therefore, absolutely necessary to define how measurements are taken before meaningful comparisons can be made.

When following a flying bat using a heterodyne detector, considerable data on pulse repetition can be collected. Bats flying straight in free space tend to use a pulse repetition rate correlated with the respiratory cycle or wing beat frequency, which in turn is related to size and flight speed. However, when bats make maneuvers or circles, e.g., when they fly in confined spaces, such as indoors or between branches, the pulse repetition rate varies with no clear regularity to interval lengths. Analyzing interval lengths from straight flight usually shows one or more distinct peaks if the number of intervals is plotted against interval length. If there is more than one peak, this can be explained by a basic rate mixed with longer intervals where pulses have been skipped. This mixture of rates forms a rhythm that can be very specific and can be used as a species "fingerprint."

Rhinolophus spp

R. ferrumequinum *R. blasii* *R. euryale*	Frequency
R. mehelyi *R. hipposideros*	Rhythm + Size & behavior

Figure 8: The CF sonar group and criteria used to identify species.

IDENTIFICATION WITHIN THE CF GROUP

There are five *Rhinolophus* species (Fig. 8); three of them, *R. ferrumequinum*, *R. blasii*, and *R. euryale*, are easily separated based on frequency alone as they have almost no overlap (Heller and von Helversen 1989; Ahlén 1990). This is easily determined using a heterodyne detector in the field, but time-expansion recordings are useful for verification and self-testing. *R. mehelyi* and *R. hipposideros* overlap in frequency but differ in pulse length (Ahlén 1988, 1990), which can be perceived with heterodyne by experienced observers. This can be confirmed by analyzing time-expanded sounds. In addition, these species differ in size and behavior (Heller and von Helversen 1989), therefore using a light or night-vision device is recommended. *R. euryale* and *R. mehelyi* overlap in frequencies in different areas of Italy (Russo and Jones 2001). The overlap is mainly between juvenile *R. mehelyi*

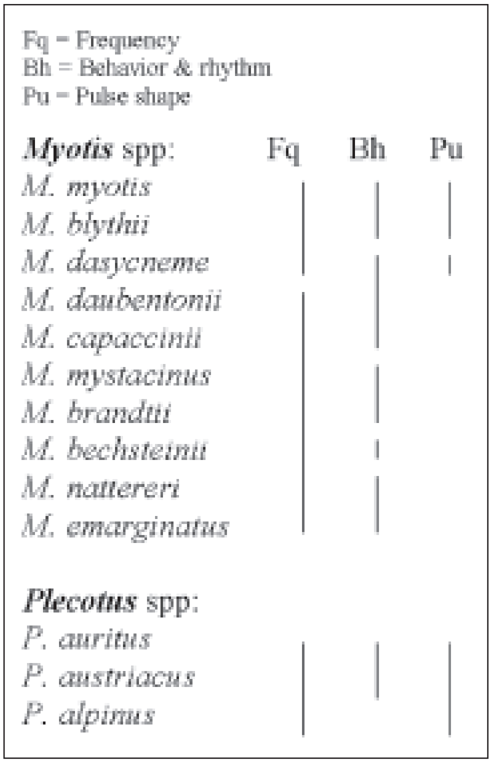

Figure 9: The FM sonar group and criteria used to identify species (or groups of species).

and *R. euryale* of all ages. Many rhinolophid bats emit lower frequencies as juveniles than as adults (e.g., Jones et al. 1992; Jones and Ransome 1993), and these age-related (and sex-related; Jones et al. 1992) effects should be considered when attempting to identify rhinolophid bats by the frequency of their echolocation calls.

IDENTIFICATION WITHIN THE FM GROUP

This group includes 10 *Myotis* and 4 *Plecotus* species (Fig. 9), which offer the most difficult identification problems in the European bat fauna. In most cases frequency can be used to separate them into groups. *M. myotis*, *M. blythii*, and *M. dasycneme* as a group have a frequency at maximum amplitude of about 35 kHz. The remaining *Myotis* species form a group with peak frequencies of about 45 kHz. However, *M. nattereri* sometimes has higher frequencies and the *Plecotus* species usually employ even higher peak frequencies (about 55 kHz). To separate them, behavior and rhythm and pulse shapes must be used. Three bats usually hunt over water surfaces – *M. dasycneme*, *M. daubentonii*, and *M. capaccinii*. *M. dasycneme*, apart from its lower frequency, can be recognized by a curved middle part to the sweep, sometimes extending to a long, almost-CF part. This is easily heard in heterodyne as drop-like sounds at about 35 kHz, but should be confirmed using time expansion recordings. The other *Myotis* species are almost impossible to identify, at least during normal field observation situations and time expansion and sound analysis are of limited help. I suggest that observers become familiar with behaviors that are specific to all of these species but only exhibited in special situations, such as hunting in natural habitats. This requires considerable experience and skill, combined with extensive periods of time to follow, listen, and watch bats hunting. When working with a species, the observer gradually becomes familiar with the relationship between behaviors and sounds. The bat's sounds indicate what it is doing. Perception and rhythm memory can provide a 'fingerprint' image of a species. It is possible to use these subtle differences to separate some difficult cryptic species, such as *Myotis brandtii* and *M. mystacinus*. The *Plecotus* species are being re-evaluated with regard to taxonomy and distribution. However, there are clear differences in behavior and pulse shape that can be used, at least to separate *P. auritus* and *P. austriacus*.

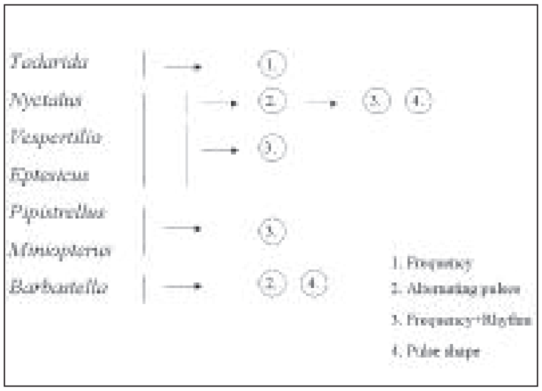

Figure 10: The QCF sonar group and criteria used to identify species.

In summary, most species in the FM group can be identified, but in surveys and monitoring where each observation is of short duration, many of these must be lumped into groups.

IDENTIFICATION WITHIN THE QCF GROUP

Seven genera represented by a total of 16 species occur in the QFC group (Fig. 10). They can all be identified, although there are some problems and pitfalls to be aware of. Two species are special and, in principal,

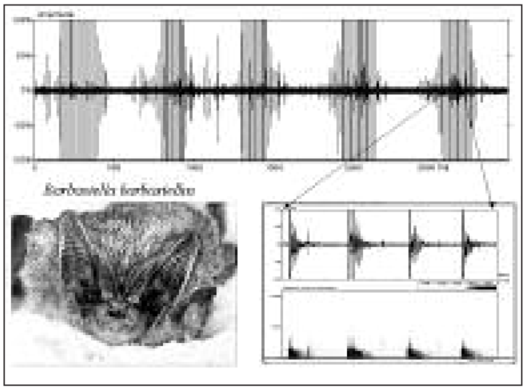

Figure 11: Heterodyne representation of *Barbastella barbastellus* sonar. In the regularly alternating rhythm, the second weak pulse is difficult but sometimes possible to detect using a heterodyne detector (see zoomed portion).

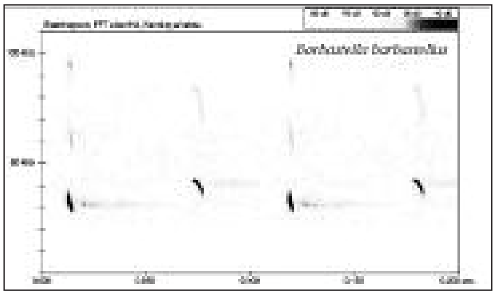

Figure 12: Time-expansion representation of *Barbastella barbastellus* sonar. The regularly alternating rhythm with a second weak pulse is easily heard using time expansion.

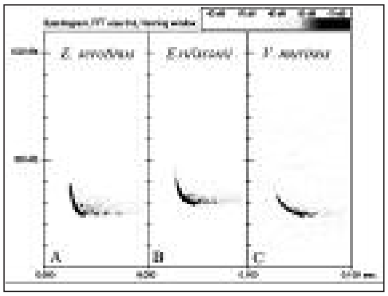

Figure 13: Single pulses of *Eptesicus serotinus* (**A**), *E. nilssonii* (**B**), and *Vespertilio murinus* (**C**) represented as sound spectrograms.

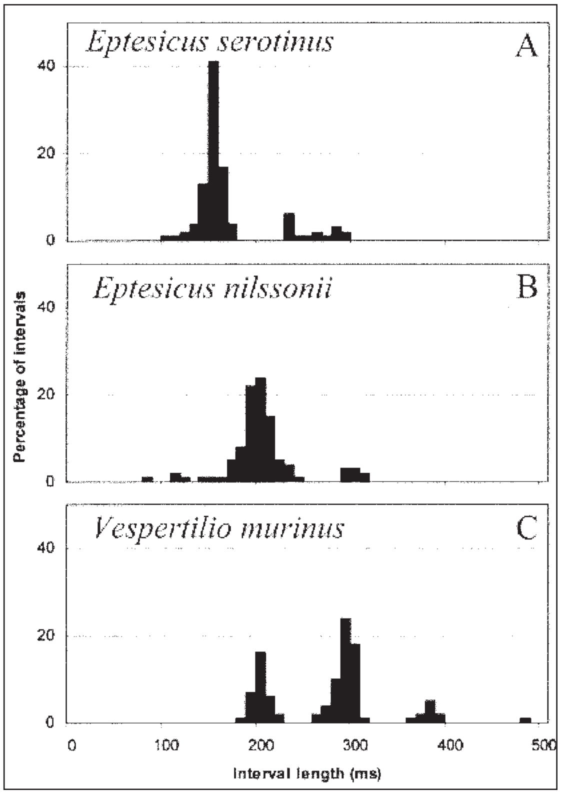

Figure 14: Pulse rhythm diagrams for *Eptesicus serotinus* (**A**), *E. nilssonii* (**B**), and *Vespertilio murinus* (**C**).

easy to identify, namely *Tadarida teniotis* and *Barbastella barbastellus*. *Tadarida* has an intense call and uses frequencies much lower than other species. *Barbastella barbastellus* is difficult to detect with heterodyne and probably impossible with a frequency division detector. The alternating pulses are more regular than in any other bat and often used during the search phase in feeding habitats (Ahlén 1981). The two pulses consist of one strong and compact pulse with peak frequency at about 33 kHz and a weak pulse that has most energy at about 44 kHz, with similar pulse intervals between pulse types. Time expansion clearly reveals this, making the calls unmistakable. With heterodyne, the sonar consists of dry clicks, but at close range a rattling or frizzling noise is produced by the second pulse type. At longer ranges, this cannot be perceived. When analyzing these "rattling" portions of a heterodyne recording using an oscillogram, it is possible to see the second pulse that follows the strong pulse. Experience suggests that *Barbastella* is difficult to identify without this knowledge, but after training, field workers quickly become skilled at finding this rare bat. I feel it is necessary to use a combination of heterodyne and time expansion to detect this species (Figs. 11 and 12).

The three genera *Nyctalus*, *Vespertilio*, and *Eptesicus*, with 8 species altogether, are easy to separate provided they are performing typical flight in free space (Ahlén 1981, 1990; Ahlén and Baagøe 1999). However, when they leave their roosts, fly among dense trees, or hunt insects around street lamps, they are difficult if not impossible to identify. This is because they vary the call repetition rate and do not call with the pulse types and rhythm that is typical and species-specific in free space (large openings or above the canopy). The same effect on vocalizations occurs when bats are released after being captured or when kept captive indoors.

To secure recordings that allow identification by instant observation or analysis, it is important to select situations when bats emit species-specific sounds and avoid situations and locations where the behavior and sound characteristics vary too much.

To identify *Nyctalus* species, the alternating pulses can be used even if they are more or less pronounced in the different species and in different situations. Frequencies and shapes of the shallowest QCF pulses are the best features to listen for or analyze. The rhythm differs between larger and smaller species.

Vespertilio and *Eptesicus* do not use regularly alternating pulses. *Eptesicus* species can be separated using the frequency of the ending QCF part which does not vary much. *Vespertilio* uses more variable frequencies, all of which are possible to identify if frequency reading or measures are combined with data on rhythm. Peaks in pulse rhythm diagrams compiled from straight flight in open air are species specific (Figs. 13, 14). Pulses are best analyzed from time expansion recordings while rhythm data are better measured from long heterodyne recordings.

Pipistrellus species can all be identified but there are overlapping features such as terminal or QCF frequency and rhythm (Ahlén and Baagøe 2001). In the case of overlapping frequencies, e.g., *P. pipistrellus* and *P. nathusii*, a difference in pulse rhythm is the key to identification. Whereas the basic rhythm is only slightly slower in the larger species, *P. nathusii*, this bat commonly uses longer intervals when hunting insects in open spaces of the forest (Figs. 15, 16). *Miniopterus* uses calls similar to *Pipistrellus pygmaeus* but can be separated on the basis of rhythm, which is a function of the bat's behavior. Confusion may occur among observers, and experience is required.

IMPORTANCE OF SOCIAL CALLS

In addition to their sonar characteristics, it is also possible to identify bats by their social calls. The advantage to using sonar is that for flying European bats it is always on. In contrast, social calls are more sporadic in occurrence. Some species emit social calls or territorial songs regularly and thereby advertise their identity. A good example is *Vespertilio murinus*, where males perform a territorial flight while repeating a complicated song, four times per second, when they fly near high buildings or alongside steep mountains (Ahlén 1981; Ahlén and Baagøe 2001; Baagøe 2001). Many, but not all, social calls are specific enough to be useful for identification (Figs. 17-20). Species identification of *Pipistrellus* species can be achieved using social calls (Ahlén 1981, 1990; Ahlén and Baagøe 1999; Jones et al. 2000; Russo and Jones 1999).

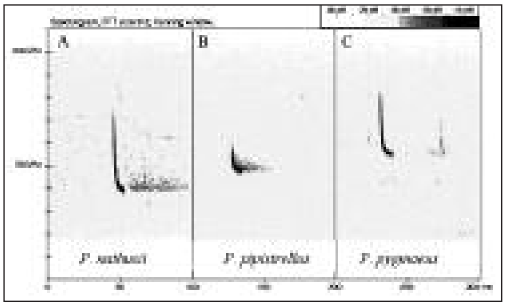

Figure 15: Single pulses of *Pipistrellus nathusii* (**A**), *P. pipstrellus* (**B**) and *P. pygmaeus* (**C**) represented as sound spectrograms.

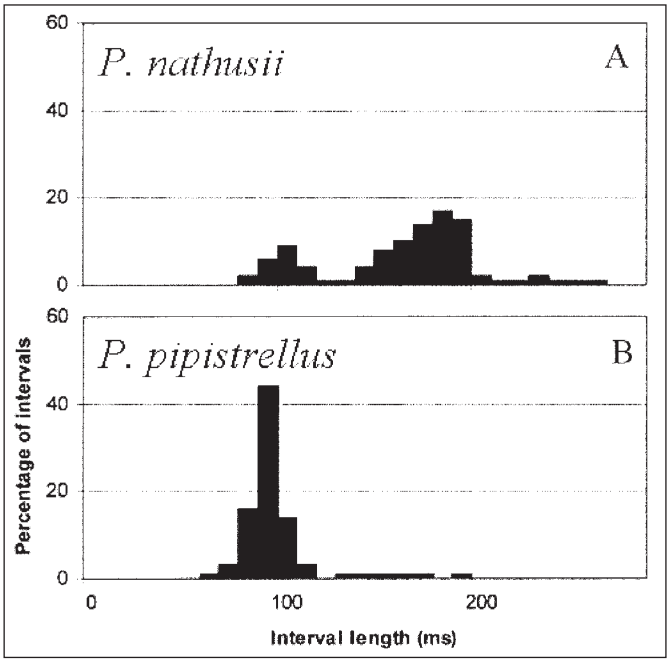

Figure 16: Pulse rhythm diagrams of hunting *Pipistrellus nathusii* (**A**) and *P. pipstrellus* (**B**).

CONCLUSIONS ABOUT HETERODYNE AND TIME-EXPANSION SYSTEMS FOR IDENTIFICATION

A heterodyne and time-expansion system is an excellent combination for field situations that require species identification. Heterodyne is a sensitive system suitable for searching for bats and allows for long-distance detection. The transformed signals provide information about the sounds for immediate perception but not for analysis. Tuned frequencies cannot be assessed exactly nor can they be saved. Heterodyne systems are useful for sampling and analyzing data on rhythm. To record and save high-quality unchanged sound segments, time-expansion systems are best. They allow both immediate identification in the field and the ability to perform subsequent analysis of recordings. The use of time expansion has enabled identification far beyond heterodyne alone and provides the best combination with heterodyne (Fig. 21). Time expansion cannot be used in real time, and another limitation is that using longer memory can block the recording of new signals during playback. Listening and recording with both systems in combination involves the use of both ears and stereo channels on the recorder (e.g., left for heterodyne and right for time expansion).

CONCLUDING REMARKS

The beginner needs to make many recordings and analyze sounds as part of the training process. With increasing experience and skill, the need to make recordings is reduced to situations when identification must be verified. This is either to test oneself or to produce direct and verifiable evidence of observations.

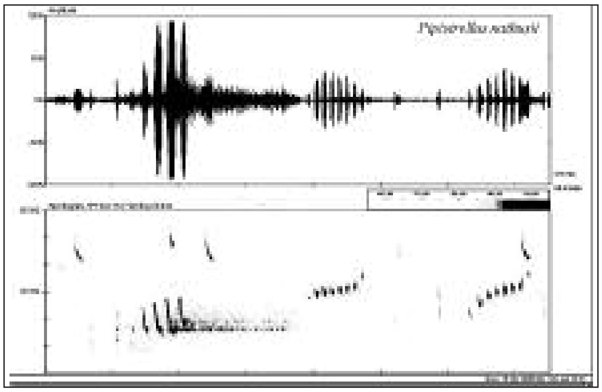

Figure 17: Territorial call of *Pipistrellus nathusii*. Three sonar pulses at about 43 kHz in the spectrogram are from a second individual.

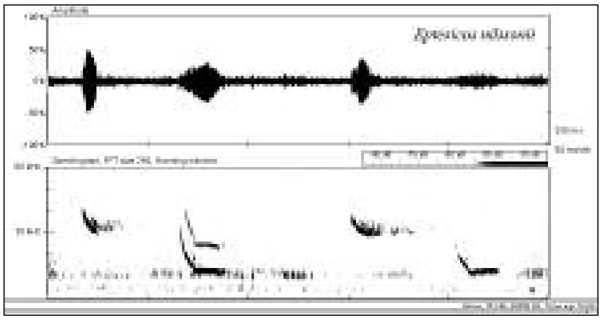

Figure 18: Social calls inserted between sonar calls of *Eptesicus nilssonii*.

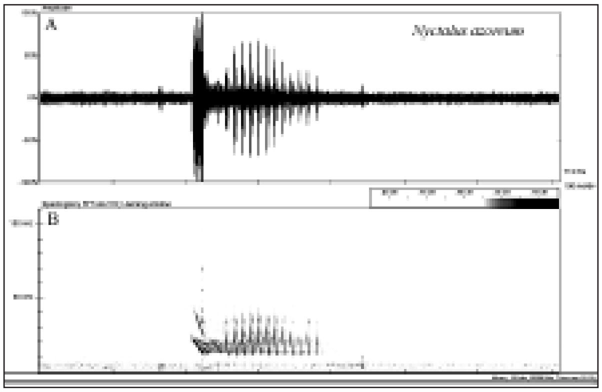

Figure 20: Territorial song of *Vespertilio murinus*.

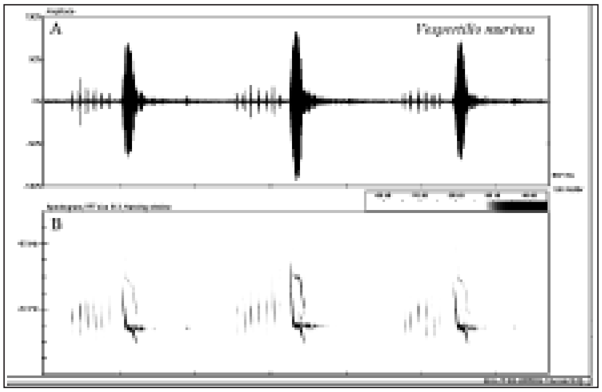

Figure 19: Social call of *Nyctalus azoreum*.

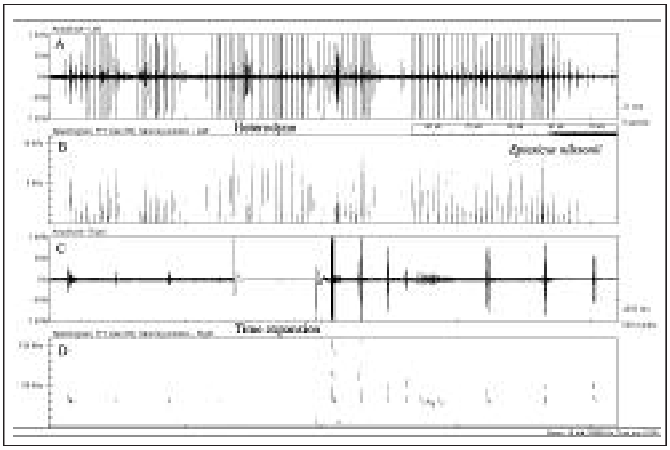

Figure 21: Heterodyne (**A**) and time expansion (**B**) of *Eptesicus nilssonii* showing how a time segment containing a buzz is triggered and replayed.

An interesting phenomenon is the fact that experienced people use criteria for identifying some species differently from the ones they teach their students. The reason is that teaching must show what is possible to measure and what differs significantly between the species. With experience, the observer becomes familiar with other "fingerprints" of a species, such as rhythm or tonal qualities, that are easily heard but more difficult to quantify or pinpoint. Still, it is possible to recognize many species immediately, in a manner analogous to the subconscious manner in which our brains enable voice recognition on the telephone.

With experience teaching the art of identifying bats under natural conditions, I have found substantial individual variation in learning ability (Fig. 22). While this may be due to variation in training or the dedication of students; innate ability and disposition are also important. The perception of subtle sound differences is a sophisticated activity that requires practice and skill. When identification of bats using ultrasound detectors started, some scientists argued that the method was not reliable. Most now dismiss this idea, but there are still different views on the usefulness of ultrasound detectors (Barclay 1999, O'Farrell et al. 1999).

Learning how to identify bats by their calls is difficult and requires more practice than to identify birds from song. Especially when beginning, it is important to work with experienced colleagues. One way to increase skill is to work in a limited area with a few known species. When familiar with those species, their sonar types and behaviors in various situations, then the study area can be expanded to where additional species occur.

Bat detector courses or workshops which combine a mixture of theory and practical training in the field will improve skills rapidly. However, continual practice and training are usually needed to maintain skills and promote self-development.

A FINAL WORD

Most European bat species can be identified acoustically using a combination of heterodyne and time-expansion detectors (Ahlén and Baagøe 1999). This method is efficient and reliable provided that the following three considerations are respected:

(1) Use the best available equipment with the highest sound quality.

(2) Sample species-specific sequences for most reliable identification.

(3) A well-developed sound memory and musical ear are prerequisites for skillful observations.

Some difficult cases, especially among the *Myotis* species, require long and careful studies until characteristic 'fingerprints' of sounds or behavior are learned. Such species must be grouped during surveys and monitoring.

ACKNOWLEDGMENTS

I thank H. J. Baagøe, L. Pettersson, and J. de Jong for valuable suggestions to an earlier version of this paper. I am also grateful for a number of improvements suggested by G. Jones, H. Limpens, and M. Brigham when reviewing the manuscript.

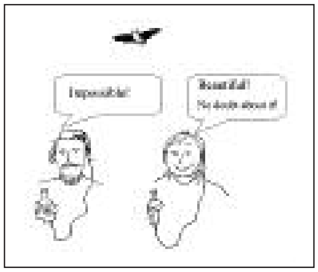

Figure 22: Individual variation in learning ability.

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DESIGNING MONITORING PROGRAMS USING FREQUENCY-DIVISION BAT DETECTORS: ACTIVE VERSUS PASSIVE SAMPLING

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Different types of bat detectors provide advantages and disadvantages for studying particular aspects of bat ecology. The Anabat II bat detector system is a widely used frequency-division detector that has the flexibility to be used for both active (researcher present) and remote (researcher absent) monitoring. Active monitoring is commonly used when recording known calls for the development of a call library, mobile sampling of transects, or when sampling near mist-netting sites. Passive monitoring permits establishment of multiple, simultaneously monitoring stations, thereby improving large-scale habitat surveys. Active monitoring results in increased call quality as researchers can follow bats with the detector, while passive sampling permits multiple simultaneous sampling stations. The type of monitoring used depends on resources (equipment and people), the length of the study, and the study objectives. Frequency-division detectors, such as the Anabat, are well suited for use in a variety of sampling designs.

Keywords: active monitoring, Anabat, echolocation, frequency division, passive monitoring, ultrasonic bat detector

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INTRODUCTION

Ultrasonic detectors have increased our ability to study bat ecology. In development of study designs, many potentially important factors need to be identified and addressed. These factors include the advantages and disadvantages of different types of bat detectors, type of sampling, objectives of the study, and resources available. Through consideration of these factors, the ability to address study objectives will be maximized.

Three broad classes of bat detectors can be used to study bat echolocation calls; heterodyne, frequency-division, and time-expansion systems. Frequency-division detectors divide the incoming frequency by a preset value to obtain a human audible representation of the call. The Anabat II bat-detector system (Titley Electronics; *www.titley.com.au*) is a frequency-division bat detector widely used in North America and Australia. Numerous authors have used the Anabat system to evaluate habitat use (Hayes 1997; Humes et al. 1999; Kalcounis et al. 1999; Krusic et al. 1996; Law et al. 1999; Mills et al. 1996; Seidman and Zabel 2001; Zimmerman and Glanz 2000) and for acoustic identification (Betts 1998; Britzke et al. 2002; Krusic and Neefus 1996; O'Farrell et al. 1999).

The Anabat system includes a broadband microphone (detects a wide frequency range simultaneously), a zero-crossings interface module (ZCAIM), and recording and analysis software. The recording software Anabat 6 has two modes: record and monitor. In record mode, calls are only saved when prompted by the user, while the monitor mode automatically records detected echolocation calls based on user-defined criteria. The Analook program permits analysis of previously recorded echolocation calls.

RECORDING MEDIA

The type of recording media used largely determines the results obtained. The Anabat system permits use of 3 different recording media. Calls can be recorded to a tape recorder. While permitting easy transport, this setup has the disadvantage of increasing analysis time as the tape must later be replayed through the computer. Tapes also have limited storage capacity and lead to reduced call quality (O'Farrell et al. 1999; E.R. Britzke, unpub. data).

Anabat also permits direct storage to a laptop computer. This setup reduces analysis time and enhances call quality, thereby enhancing the ability to acoustically identify species (O'Farrell et al. 1999). However, use of a laptop computer requires additional equipment (batteries, inverters, protective boxes, etc.). Because Anabat files are small (1-15 kb each), a 200 MB hard drive contains sufficient storage for extensive sampling over multiple years (Corben and Fellers 2001).

Recently, the CF Storage ZCAIM has been developed that merges the benefit of the two recording media for use in passive monitoring. This component saves calls to a compact flash card. Digital storage of the call means that quality is as high as with a laptop, but less equipment is necessary.

The recording situation determines the best recording media to be used. For example, if the researcher is present at the recording station, benefits from the ability for real-time analysis possible with the laptop computer are probably preferred.

COMPARISONS WITH TIME-EXPANSION DETECTORS

The Anabat system eliminates information about harmonic structure by only using the harmonic with the most energy. Additionally, the Anabat system does not retain information about call amplitude. In comparison with time-expansion detectors, frequency-division detectors measured higher minimum frequencies and lower maximum frequencies (Fenton et al. 2001). However, differences between the two systems amount to 1-2 kHz, which is smaller than many other sources of variation (Murray et al. 2001).

Several studies have compared the number of calls recorded in direct comparisons of the 2 detector systems. The Anabat system detected fewer echolocation calls than did a time-expansion detector (Fenton 2000; Fenton et al. 2001), but these studies focused on the detection limits of the two bat detectors. When sampling in the field, time-expansion detectors experience a "dead time" as calls are being downloaded to tape (although see Jones et al., this volume), thereby effectively sampling for only 7.5% of the time (Fenton 2000). Even with the difference in sensitivity between the two systems, frequency-division systems will detect more than twice the number of files (Corben and Fellers 2001). In a large-scale field comparison in forests of the southern United States, the differences in number of files recorded between the 2 systems varied with sampling location. The Anabat system detected more bat activity at ground level, but the reverse was true above the canopy (M. Menzel, unpub. data). To maximize applicability of results, as many recording situations as possible should be incorporated into comparisons of detector types.

CALIBRATION

Microphones used in different types of ultrasonic detectors differ in their sensitivities to high-frequency sounds. Differences in microphone sensitivity also occur within the same type of bat detector (Larson and Hayes 2000). Therefore, before commencing any study using multiple bat detectors, equipment must be calibrated (Hayes 2000). This requires an ultrasonic sound source (e.g., insect repeller – Larson and Hayes 2000). Calibration should also be done periodically throughout the study. Additionally, detectors should be randomly assigned to recording stations to minimize the impacts of differing microphone sensitivities on the results.

TYPES OF MONITORING DESIGNS

Bat detectors allow 2 types of recording: active and passive monitoring. Active monitoring involves the researcher being present to adjust the orientation of the microphone relative to the bat and to manually save detected echolocation calls. Active monitoring permits contact to be maintained with the bat, resulting in improved call quality (Fig. 1). Passive monitoring involves the automatic recording of echolocation calls without an observer present, based on predefined criteria. The directionality of the microphone in a fixed (passive) position can exert a strong influence on sound quality and on the quantity of calls recorded. Thus, a tradeoff exists between call quality and the benefits of simultaneous sampling.

Studies using passive recording generally include multiple systems spaced across the landscape, thereby requiring protection of equipment from the environment. Protective boxes have been developed for both tape recorder (Hayes and Hounihan 1994) and laptop-operated (O'Farrell 1998) systems. While these setups serve to protect the equipment from weather, factors such as humidity have a potentially major influence on the detection of echolocation calls.

EXAMPLES OF USE

The objectives of the study determine the most effective sampling design. Below are examples of sampling design most useful for examining species identification, locating high-activity areas, acoustic sampling in conjunction with capture techniques, and evaluating habitat use.

Species Identification

Before acoustic identification can be attempted, a known-call library must be established. Known calls are commonly collected from active recording of hand-released bats. However, immediately after release, bats generally produce atypical calls (i.e., distress calls – Britzke et al. 2002; O'Farrell et al. 1999). Additionally, call sequences can be examined after the release of some individuals to determine if researchers need to adjust their distribution around the release point to increase recording quality. Passive monitoring, while still permitting species identification, inflates the number of calls that cannot be identified (E.R. Britzke, unpub. data).

Locating Areas of High Activity

Another use of acoustic monitoring is to find sites with high activity during initial surveys of large areas. Commonly, research is focused on the distribution of a target species within a large area (i.e., national forest, national park, etc.). Within a large area, the number of potential sites is generally greater than the number that can be sampled during the study. Using active monitoring, sites with high levels of activity can be located or the presence of a target species can be determined. Numerous areas can be surveyed for short periods of time to generate a representative picture of species present at a site, and later analysis can establish a sampling priority for these sites. Thus, effort can be focused on areas with increased likelihood of locating target species. For example, in the southern United States, active monitoring was used to survey 40 kilometers of roadways in a single night for the federally endangered Indiana myotis (*Myotis sodalis*). One site was identified and subsequent mist netting led to location of the second (and largest-known) Indiana myotis maternity colony in Tennessee (Harvey 2002).

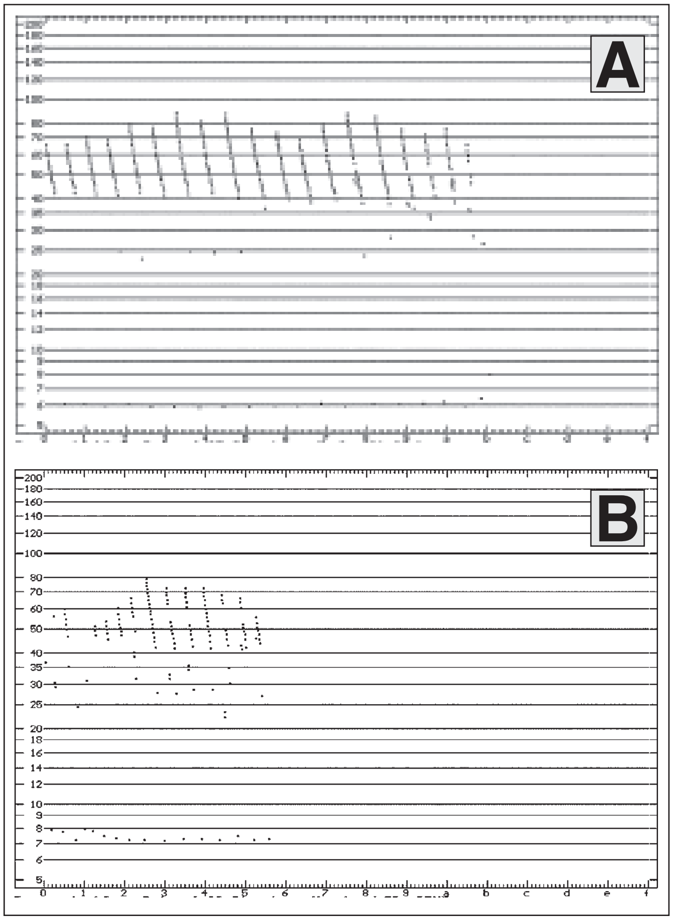

Figure 1: Time-frequency displays generated by Analook of northern long-eared myotis (*Myotis septentrionalis*) search-phase calls recorded using active (**A**) and passive recording (**B**). Time between calls has been compressed to allow multiple calls to be displayed simultaneously. Time (msec) is on the x axis, and frequency (kHz) in on the y axis.

Additional Sampling

Ultrasonic detectors are commonly used in conjunction with traditional capture techniques. Active monitoring can also be used to sample when the researcher is not monitoring mist nets. In areas where the majority of the species use high intensity echolocation calls, simultaneous sampling with the Anabat system detected the presence of more species than mist nets (Murray et al. 1999; O'Farrell and Gannon 1999). However, in both of these studies, the most complete picture of the bat community was uncovered when ultrasonic detectors are used in conjunction with mist nets. Using a variety of methods maximizes the probability of detecting all species in an area.

Habitat Use

As bat populations decline throughout the world, understanding habitat use is becoming increasingly important. While data from bat detectors cannot be used to determine the number of individuals present in the area, there are several methods for defining the amount of bat use in an area. The most common measure of bat activity is the number of files or passes (Fenton 1970; Hayes 1997; Krusic et al. 1996). Britzke et al. (1999) developed a measure that incorporates the length of the echolocation call into levels of bat activity. Miller (2001) described an activity index that quantifies activity by the number of time periods that a species is present. Bat activity should be used as a relative term for assessing bat use. In some areas, 30 passes may represent high activity, while in others 100 may represent a low activity level. No matter the technique used, the researcher must explicitly define the means by which bat activity is measured and discuss the implications of this definition on the results.

Two sampling methods have been employed to infer habitat use by bats. Different habitat types can be sampled actively as an observer moves along a transect (Mills et al. 1996; Zimmerman and Glanz 2000). To account for temporal variation in bat activity, each transect is sampled during different periods of the night. However, the majority of the studies designed to examine habitat use have employed passive monitoring (Humes et al. 1999; Kalcounis et al. 1999; Krusic et al. 1996; Seidman and Zabel 2001). In these studies, a detector is randomly placed in each habitat type being sampled, and activity is compared among habitat types to infer habitat use. Due to considerable among-night variation in activity, sampling should be done simultaneously to increase comparability (Hayes 1997).

While numerous studies have examined habitat use, there is no published literature about spatial variation within a habitat (Hayes 2000). If spatial variability is not constant among habitat types, the results of studies examining habitat use are of limited value. Variability would be expected to increase with structural complexity of habitats as complex habitats have isolated areas that are suitable for bat activity, while less complex habitats (i.e., open fields) have one large area suitable for bat activity. Future studies need to examine the relationship between structural complexity and spatial variation in bat activity. This information can be used to improve the design of studies exploring bat habitat use.

The type of sampling design depends on many factors. Frequency-division bat-detector systems are useful in both active- and passive-monitoring schemes. Additionally, call libraries have been developed for large areas using the Anabat system, thereby permitting acoustic identification. Overall, the flexibility of the Anabat system in sampling permits this system to be used in a large number of studies of bat activity and habitat use.

ACKNOWLEDGEMENTS

I thank U.S. Forest Service, U.S. Fish and Wildlife Service, and the Center for the Management, Utilization, and Protection of Water Resources and Department of Biology at Tennessee Technological University for financial support. C. Corben provided valuable assistance with the Anabat system. Many people assisted with my research on ultrasonic detectors, including: R. Currie, M. Harvey, S. Loeb, J. MacGregor, K. Murray, and L. Robbins.

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DESIGNING BAT ACTIVITY SURVEYS USING TIME EXPANSION AND DIRECT SAMPLING OF ULTRASOUND

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We reviewed acoustic studies that use time-expansion methods to determine habitat use by bats in Europe. Species identification can be quantified by using discriminant function analysis or neural networks. These methods maximize the information recorded from echolocation calls and allow confident classification of calls to species. Because the recording equipment is expensive, surveys typically involve one recording device and mobile sampling along transects. We walk transects for a fixed time period, starting at a fixed time after sunset. Bats are detected by listening on frequency-division mode, and calls are time-expanded upon detection. Our methods involve sampling replicates of each habitat and visiting habitats in random order over the summer. We estimate foraging success by calculating the ratio of feeding buzzes to passes. We illustrate our methods by describing habitat surveys in Britain and southern Italy. The broad bat community in Italy presents considerable challenges for acoustic identification, but nevertheless we achieved a high rate of correct classification of calls to species. More recently, we have used paired sampling of organic versus conventional farms, together with direct sampling of ultrasound, to determine whether intensive farm management has a detrimental effect on bat activity. Direct sampling overcomes the wasted download time inherent in time expansion, and allows acquisition of extended high-quality recordings.

Key words: acoustic identification, bat activity, bat detectors, habitat use, ultrasound
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INTRODUCTION

Being nocturnal, bats are difficult animals to survey visually. Because many species have distinctive echolocation and social calls, there has been increasing interest on developing acoustic surveys of their activity (e.g., Kalkounis et al. 1999; Seidman and Zabel 2001; Vaughan et al. 1997). Survey methods should rely on a robust method for acoustic identification. Although some authors have taken a qualitative approach to acoustic identification of bat species (O'Farrell et al. 1999), we agree with Barclay (1999) and argue that acoustic surveys must be quantitative and objective. Objectivity is especially important to control for differences in identification abilities among recorders, as well as when surveys are to be repeated in the future (for example, to assess long-term changes in bat activity). Here we describe some methods and results from studies of bat activity using time expansion detectors. Important assumptions relating to studies of bat activity by acoustic monitoring (e.g., relating activity to habitat quality, whether feeding buzzes accurately reflect foraging activity) are reviewed by Hayes (2000). These assumptions relate to all detector methods.

RECORDING: TIME EXPANSION AND DIRECT SAMPLING

Several types of detector have been used for acoustic surveys. Walsh and Harris (1996) described the use of heterodyne detectors by volunteers in a large-scale survey of bat activity in relation to land class in the United Kingdom. Heterodyne detectors have several drawbacks, one of the most important being their restricted bandwidth for detecting ultrasound (typically ± 5-8 kHz around the tuned frequency). Frequency division has been widely used in bat surveys, especially in the United States where Anabat detectors are popular (e.g., Lance et al. 1996; Murray et al. 1999). We have used time expansion and direct sampling of ultrasound to survey bats in Europe. This is partly because we are interested in describing (and classifying) echolocation calls of bats with as little information loss as possible. Moreover, time-expansion detectors are more sensitive than frequency-division models (Fenton et al. 2001) partly because they use microphones that are more sensitive across a broader bandwidth. Time-expansion detectors therefore detect more calls per unit time and presumably at greater distances (Fenton et al. 2001). However, because calls cannot be recorded while call sequences are being downloaded to recording media (typically 20 s for downloading a 10x expanded 2 s sequence of ultrasound), considerable sampling time is wasted when using time expansion.

A major advance in recording methods involves direct sampling of ultrasound to computer hard disks, whereby fast sampling PCMCIA (Personal Computer Memory Card International Association) data-acquisition cards can be used to sample ultrasound without encountering aliasing problems (Pettersson 1999). Direct sampling allows minimal information loss from signals, high sensitivity, and continuous recording for long time periods (e.g., one hour with 16-bit resolution and a 2.4 Gb hard disk). Because direct sampling involves use of a laptop computer in the field, it is sometimes more practical to carry a small time-expansion detector linked to a tape or DAT recorder. We will therefore describe how both time-expansion and direct-sampling methods can be used in surveys of bat activity. We start by describing methods of acoustic identification and will cover aspects of survey design. Finally, we will describe results from some case studies of acoustic monitoring of bats in Europe.

ANALYSIS: DISCRIMINANT FUNCTION ANALYSIS AND NEURAL NETWORKS

The first stage in developing an objective method for acoustic surveys is to record and analyze echolocation calls to develop a call library from species in your study area. Bat echolocation calls exhibit considerable intraspecific variation because of the effects of acoustic clutter on call design (Schnitzler and Kalko 1998) and through inter-individual variation related to age, gender, and morphology (reviewed in Jones et al. 2000). We argue that it is best to be conservative in assessing the degree of species identification by recording the study species in as many ecological circumstances as possible. This means recording bats in clutter, in open habitats, and even when exiting roosts. If acoustic surveys are based across a wide range of habitats, it is important that call variation in relation to habitat be taken into consideration. Information on features such as distance of the bat to clutter, position of the microphone relative to the bat, and so forth can be invaluable. It is also important to record a large number of individuals (not calls), as calls from individual bats should be used in statistical analyses to avoid pseudoreplication. In our studies, we select one call per bat for analysis (Parsons and Jones 2000; Vaughan et al. 1997).

Once a call library is available, it is possible to assess the reliability of acoustic identification to species. Temporal (e.g., pulse duration, pulse interval) and frequency (e.g., highest and lowest frequency, frequency of most energy) parameters are extracted from calls for multivariate analysis. It is important to realize that frequency-divided output may not give an accurate measure of call duration (Fenton et al. 2001).

Techniques such as discriminant function analysis (DFA) (e.g., Krusik and Neefus 1996; Lance et al. 1996; Vaughan et al. 1997; Zingg 1990) and neural networks (Parsons and Jones 2000) can be used to classify calls made by different species according to multivariate analysis of call parameters. Neural networks may achieve even higher rates of correct classification to species than discriminant function analysis (Parsons and Jones 2000).

The most problematic species to discriminate acoustically in temperate regions are often bats in the genus *Myotis*, many of which produce brief, broadband frequency-modulated (FM) calls. Nevertheless, some *Myotis* species can be identified with confidence, often through differences in starting and ending frequency, and bandwidth (Parsons and Jones 2000). Using time-expanded calls, Vaughan et al. (1997) correctly classified 67% of calls from four *Myotis* species and *Plecotus auritus* by using discriminant function analysis (random classification would have been 20% correct). Parsons and Jones (2000) were able to identify 82% of 5 *Myotis* species correctly by using artificial neural networks, with classification rates for individual species varying between 75% (*M. daubentoni*) and 90% (*M. nattereri*).

Multivariate methods have been used successfully for species identification from time-expanded calls even in bat communities where species richness is high. The analyses of Vaughan et al. (1997) and Jones and Parsons (2000) dealt with 13 and 12 species respectively in the United Kingdom. Russo and Jones (2002) recently applied DFA to calls from 18 Italian species, and obtained a correct classification rate of 82%. In developing methods for acoustic identification, typically species that can be identified unambiguously from call structure are removed (e.g., several rhinolophid species, and the low frequency – ca. 11 kHz – echolocator *Tadarida teniotis* in Italy) to restrict the discriminant function analysis to species with similar calls. Always check that the assumptions of discriminant function analyses are met (sometimes quadratic, rather than linear analyses must be used), use cross validation, and consider specifying prior probabilities (sample sizes).

The manner in which output from multivariate analysis is handled must be considered. A cutoff degree of certainty in identification can be specified so calls that are not classified with a specified degree of confidence are regarded as 'unclassified.' Having a known degree of confidence in certainty of identification is worthwhile, and some researchers may wish to limit analyses to calls that lie in areas of multivariate space where identification is unambiguous. We prefer not to do this, because call designs associated with particular habitat features (especially clutter) may have to be removed from the analysis. Consequently, the number of times that a particular species is scored as being present in habitats where identification is not absolute would be underestimated. Indeed, the effect of habitat features (e.g., foliage) on detectability of echolocation calls requires further research.

SURVEY DESIGN

Active versus remote monitoring

We prefer active monitoring for two reasons. First, equipment for recording time-expanded echolocation calls is expensive, so the purchase of several units necessary for most remote-monitoring studies is often not feasible. Second, active monitoring maximizes encounter rates with bats, whereas a remote unit placed at one site may repeatedly record the same individual animal. We therefore walk transects through habitat patches. Bat detectors are used in frequency-division mode; so all frequencies used by bats in our study areas can be detected. When a bat pass is heard, time-expanded sequences are tape recorded for species identification. Vaughan et al. (1997) recorded in stereo from two detectors, one set to time expansion, the other to frequency division.

Two 1-km transects were walked for 45 mins at a fixed walking speed, starting 30 mins after sunset. Each transect was confined to one land-use type. Meteorological data were recorded, and transects were not walked in heavy rain because of the risk of equipment damage.

Surveys may also combine active and static monitoring, whereby a series of different sites are monitored for a fixed time each over one night. We adopted this approach in our study of bat activity on organic versus conventional farms (see below). It is also worth considering whether transect features (e.g., presence of a footpath through woodland) are likely to bias the chances of encountering bats.

Replication and randomization

Vaughan et al. (1997) recorded bat activity in 10 different land-use types. Three replicates of each were studied, with a minimum distance of 5 km between replicates. Each site was visited 3 times, once before the main period of lactation, once during lactation, and once after most bats had finished lactating. With multiple visits to the same transect in different seasons, transects were walked in the same direction so that order effects were standardized. Sites were visited in random order within each block of 10 land-use types in each season. This approach allowed analysis using ANCOVA (analysis of covariance), after transformation of bat-pass data to achieve normality. Site was nested within land-use type,

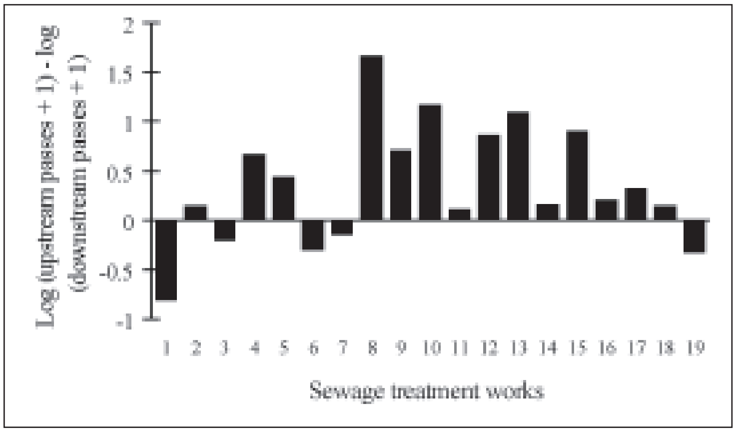

Figure 1: Results from a study of bat activity using paired sampling. Differences in log bat passes represented as values upstream minus downstream of sewage outlets at 19 sewage treatment works. Both measures show higher upstream values (see Vaughan et al. 1996).

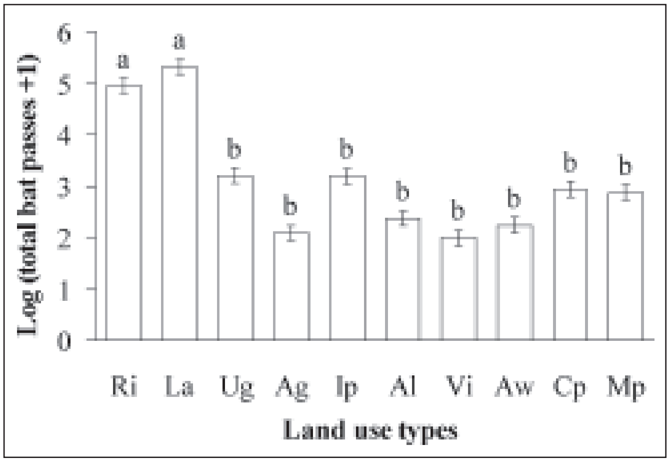

Figure 2: Surveys of bat activity in relation to land-use type in England indicate that bat activity is highest over rivers and lakes. Bars are adjusted mean log transformed counts of bat passes recorded in 10 land-use types (Rivers (Ri), Lakes (La), Unimproved grassland (Ug), Amenity grassland (Ag), Improved cattle pasture (Ip), Arable land (Al), Villages (Vi), Ancient semi-natural woodland (Aw), Conifer plantations (Cp), Mixed plantations (Mp) – habitats defined in Vaughan et al. (1997b)). Groups of habitat types supporting activity levels, which are not significantly different from one another, are indicated by the same letter. Bars represent the means of nine transects (three sites visited three times each) with standard deviations shown.

season was a crossed factor, and temperature was a covariate. This is effectively a repeated-measures analysis of variance (ANOVA), with each season considered as a repeat.

Russo and Jones (2003) used a similar approach in a study of bat activity in relation to habitat type (10 categories) in southern Italy, but increased the number of replicates for each habitat type to 6, and visited each site only once. Future studies could incorporate species accumulation curves to determine the optimum number of transects walked in each habitat (see Walsh et al., this volume). In both of our studies (Russo and Jones 2003; Vaughan et al. 1997), we used post-hoc tests (Bryant-Paulson Tukey tests) on adjusted means (effects independent of the covariate) to determine which habitats differed from one another in terms of bat activity. Activity was measured by monitoring the numbers of bat passes. An index of feeding activity relative to searching for prey was calculated as the ratio of feeding buzzes to bat passes.

Paired sampling

If 2 habitats or situations are being compared, paired sampling is a powerful technique because it controls for variation in bat activity due to environmental factors. Vaughan et al. (1996) used paired sampling (in this case while recording frequency-divided calls) to investigate the effects of water quality on bat activity. In this study, sewage output was used as a surrogate measure of water quality. Two people simultaneously sampled for bats at sites upstream and downstream of sewage outputs. Sites upstream and downstream from 19 separate sewage outputs were sampled to achieve statistical power, and Wilcoxon signed rank tests were used to test whether the difference between the number of passes upstream and downstream differed significantly from zero (Fig. 1). Higher activity occurred upstream in 14 of 19 pairs, and significantly more bat activity occurred upstream compared with downstream.

Currently, paired sampling is being used to determine the effects of agricultural intensification on bat activity, testing the hypothesis that bat activity is higher on organic farms than on conventional farms (L. P. Wickramasinghe, pers. comm.). If agricultural intensification has had a detrimental effect on bats, we predict that activity will be higher on organic farms where many methods of intensification (hedgerow removal, use of pesticides, and artificial fertilizers) are absent.

The project involves direct sampling of ultrasound (after detection by frequency division) at sample points within habitats using a paired-site design. Detailed habitat surveys are conducted to match pairs of farms that are as similar as possible to one another, with the exception of farm management, one farm using conventional farming methods and the other being farmed using organic methods (as defined by The Soil Association, United Kingdom, see *http://www.soilassociation.org/sa/saweb.nsf/standards/index.html*). Paired farms of similar sizes were no more than 5 km apart, which controlled for geographic varia-

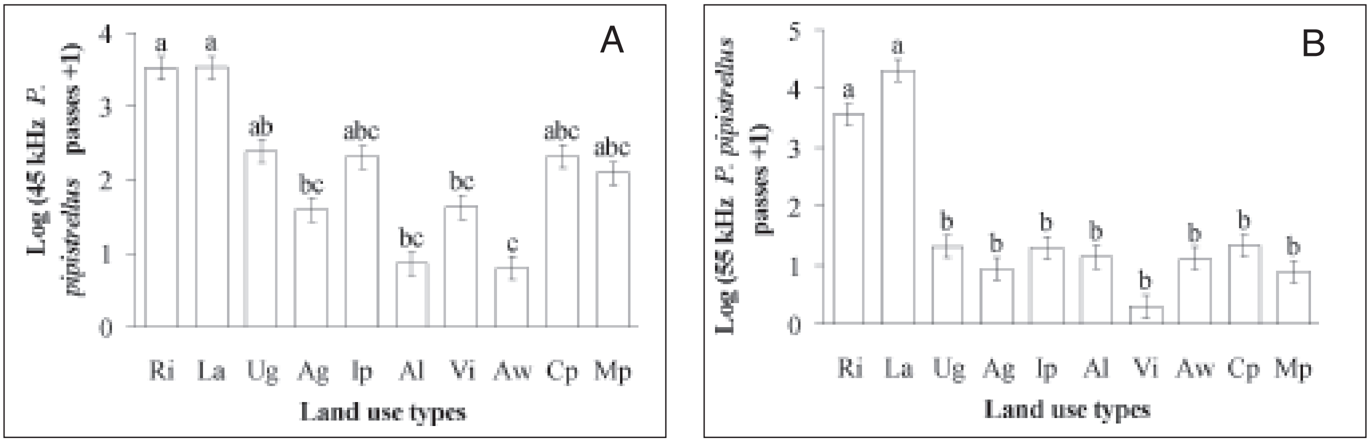

Figure 3: Two cryptic species of pipistrelle exhibit different patterns of habitat use. Bars are adjusted mean log transformed counts of passes for (**A**) the 45 kHz phonic type of *P. pipistrellus* (suggested name *P. pipistrellus*) and for (**B**) the 55 kHz phonic type (suggested name *P. pygmaeus*). Abbreviations and conventions as in Fig. 2. Figure from Vaughan et al (1997b).

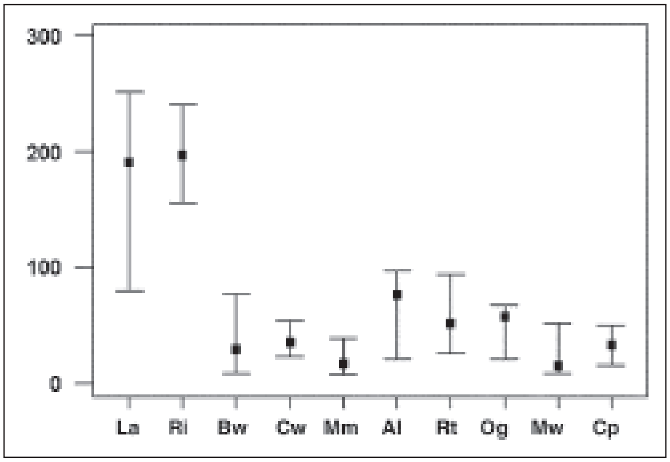

Figure 4: Median and interquartile range of bat passes recorded (all species) in 10 habitats in southern Italy. Habitats are Lakes (La), Rivers (Ri), Beech woodlands (Bw), Chestnut woodlands (Cw), Mediterranean macchia (Mm), Arable land (Al), Rural towns (Rt), olive groves (Og), Mediterranean and sub-Mediterranean woodlands (Mw) and Conifer plantations (Cp).

tion. Four comparable habitats were selected for sampling at each farm pair (pasture, arable, water, woodland), with each habitat type being extensive enough for 3 sampling points at least 15 m from each other. The order of habitats sampled within a pair was the same, but visitation of habitat types between pairs was randomized. Both sites within a pair were sampled on consecutive nights, and recording commenced 1 h after sunset. Although simultaneous sampling of sites would control for night-to-night variation, the nature of the equipment being used made this impossible. Sites within a pair were therefore matched for weather conditions, and temperature differences had to be within 4 degrees of when the first site was sampled. Other environmental variables (e.g., wind speed) and habitat-structure variables were measured at each sample point and included in the analysis.

Paired-sampling methods can still be undertaken even if there are more than 2 treatments to compare by employing repeated-measures ANOVAs. Paired-sampling and repeated-measures designs increase statistical power compared with unpaired designs by separating variability among treatments from variability among replicates. Ninety-five percent confidence intervals are thus reduced, making it easier to detect differences between treatments.

CASE STUDIES

Acoustic surveys of habitat use by British bats revealed that once total bat activity was adjusted for air temperature, activity (of all species combined) was significantly higher over rivers and lakes than over other land-use types investigated (Vaughan et al. 1997, Fig. 2). Especially interesting was a difference in habitat use by 2 recently described cryptic species of pipistrelle, discovered by analyzing differences in echolocation calls (Jones and van Parijs 1993). The '45 kHz phonic type' (*Pipistrellus pipistrellus*; Jones and Barratt 1999) was a generalist in habitat use. Although this species was detected most frequently over rivers and lakes, activity was also common in most other habitats surveyed (Fig. 3a). Conversely, the '55 kHz phonic type' (*P. pygmaeus*; Jones and Barratt 1999) concentrated its activity near lakes and rivers (Fig. 3b). These patterns of habitat use are consistent with dietary studies, which suggest that *P. pygmaeus* feeds on insects with aquatic larvae more than *P. pipistrellus* (Barlow 1997).

Habitat surveys of a similar design to those of Vaughan et al. (1997) were conducted in Mediterranean habitats in southern Italy (Russo and Jones 2003) in a landscape affected by human activity for over 300 generations (Blondel and Aronson 1999). Bat activity was most frequently recorded over rivers and lakes (Fig. 4), confirming that these habitats are important bat foraging areas over a wide geographic scale.

Given that riparian habitats are important over a wide geographic area (see also Racey 1998), is it possible to identify particular landscape features along rivers that are especially important for bats? Warren et al. (2000) used time-expansion detectors to investigate the distribution of *M. daubentoni* and *P. pipistrellus* in relation to small-scale variation in riverine habitat. Eight 1.5-km transects were walked at constant speed along a 13-km stretch of river, starting 30 min after sunset. Both species were most active along stretches of the river with smooth water surfaces and with trees on both banks. Smooth water surfaces facilitate the detection of prey on water surfaces by echolocation (important for *M. daubentoni*), and stretches of river with trees on both banks had more insects associated with them than stretches without trees (Warren et al. 2000, Fig. 5).

CONCLUSIONS

We have illustrated some of the ways in which time expansion and direct sampling can be used in studies of habitat use by bats. Our focus has been on riparian habitats. We show that rivers and lakes support higher levels of bat activity than other habitats studied in Britain and Italy. Water quality can affect bat activity, and tree lines can increase the value of riparian habitats for bats.

Time expansion and direct sampling are the only methods suitable for accurate descriptions of acoustic parameters of bat echolocation calls (Fenton 2000; Fenton et al. 2001). However, time-expansion detectors and direct sampling of ultrasound have been used in relatively few surveys of habitat use by bats. More research has been conducted with heterodyne and frequency-division equipment. The expense of time-expansion detectors and the loss of recording time during downloading of call sequences are 2 drawbacks of this methodology. However, bat detectors linked to time-expansion devices record more bats per unit of recording time than do some frequency-division detectors (Fenton 2000; Fenton et al. 2001). The high sensitivity and relatively flat frequency response of some of the microphones supplied

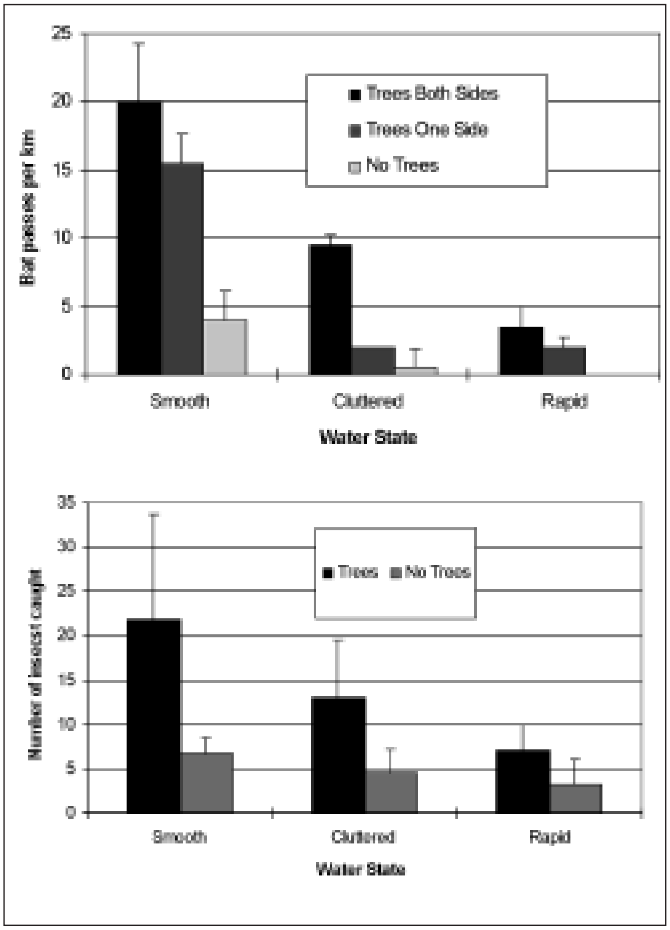

Figure 5A: *Pipistrellus pipistrellus* has highest activity along stretches of river, which are smooth and have trees on both banks. **5B:** Highest insect densities are recorded along river stretches that are smooth and tree-lined. Means + SDs are illustrated. Figure from Warren et al. (2000).

with the best time-expansion detectors are important specifications for scientists interested in recording sounds from species that emit calls of low intensity and/or high frequency. Direct sampling overcomes problems of lost recording time. The increased information content of calls recorded by time expansion and direct sampling is also likely to result in better discrimination of species with similar call structure. For example, preliminary results suggest that *Myotis* species may be discriminated more confidently using time-expanded calls than by frequency-divided recording (L.P. Wickramasinghe, in litt.). Therefore direct sampling, with its advantages of high sensitivity and retention of maximal information content of calls, offers great benefits for future studies of habitat use by bats. In choosing bat detectors, trade-offs between recording quality and cost are inevitable (Fenton 2000), and the high quality offered by the recording techniques described in this paper may not be necessary for all studies of habitat use, although they are essential for describing the echolocation calls of bats (Fenton et al. 2001).

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FREQUENCY DIVISION: A TECHNICAL OVERVIEW

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Frequency division bat detection is usually equated with the Anabat system. However, it is perhaps more useful to discuss frequency division technology outside the context of Anabat. This permits a better evaluation of the true strengths and weaknesses of this technology. In this context, it can be fairly asserted that frequency division has significant value when applied to the acoustic survey of bats and bat activity. Frequency division technology provides access to robust field equipment, and an economical means of surveying large areas over long periods of time.

Key words: bat detectors, echolocation, equipment, frequency division, technology

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In order to set a foundation for discussing frequency-division technology, it is important to make two points clear. The first point is simply that discussion of frequency division is not a discussion pertaining strictly to Anabat detectors. Even though Anabat detectors represent a significant use of frequency division, similar technology is employed in many bat detectors. Secondly, this discussion centers on the frequency-division technique in its simplest form, without the employment of peripheral techniques to restore or preserve amplitude information. Circuits used to preserve or restore amplitude information in frequency-divided modes many times add noise and other artifacts that would be considered detrimental to many of the benefits provided by simple frequency division.

A frequency-division detector is somewhat harsh to listen to, compared to other bat detector technologies, such as heterodyne or time expansion. This is due in part to the high harmonic content of full amplitude square waves, which are normally produced by a simple frequency-division circuit. It is also the result of compressing an entire 160 kHz or more bandwidth into a real-time audible signal. The structurally richer and more pleasing sounds produced by heterodyne and time-expansion methods are generally easier for the human ear to interpret and identify, but they do so at a cost. The premium paid for employing the heterodyne process is limited bandwidth. The time-expansion technique excludes the ability to do real-time correlation between observed activity and monitored acoustics, and has the further limitation of only being able to sample some of the acoustic activity present.

One perceived drawback of frequency division is that of a lack of sensitivity. This is not the simple issue that many think it is. Heterodyne detectors are indeed more sensitive when used for active monitoring. This is not due to superiority in transducers or amplification circuits, but the fact that the human brain, which excels in extracting significant information in a fragmented and noisy environment, further processes the heterodyned signals, thus allowing a successful outcome from what might otherwise be considered a substandard signal. The circuits used in frequency-division detectors can be as sensitive as equivalent heterodyne circuits, but they are only useful if used with operating thresholds set above the background noise. When considering sensitivity, one must also consider the resultant signal quality, background noise, and the usefulness of the overall result.

Another often-discussed characteristic of frequency division is the lack of amplitude information in the output signal. While this can work against someone trying to determine the frequency of greatest power in a bat call, or attempting to undertake detailed call-structure analysis, it will not adversely affect survey and analysis techniques that do not require amplitude information. Frequency and timing elements, which are preserved and extracted by frequency division, are the two most fundamental elements of bat echolocation call analysis. There is actually a benefit to the manner in which a frequency-division circuit deals with variation in amplitude. It functions equally well in the presence of both strong and weak signals, as long as the sounds themselves are above the detection threshold level. Full spectrum technologies using amplitude-inclusive spectral analysis must have their sensitivity settings carefully monitored. Otherwise, the signal may be too low, becoming lost in the background or system noise - or too high, creating spurious harmonics or other forms of distortion which render proper analysis difficult, if not impossible. The sensitivity threshold setting of a frequency-division detector is much less critical due to the nature of its circuitry.

Advantages of frequency division are often overlooked, or perhaps, simply not appreciated. A frequency-division circuit is inherently stable, requiring only a few components to accomplish its function. A small number of components means a minimal number of failure points. Heterodyne and time-expansion circuits are far more complex in nature, employing critical timing elements that greatly affect the accuracy of the signal detected and recorded. Environmental factors, such as ambient temperature, can affect these timing circuits and degrade the data collected. This sets frequency division apart as the more robust technology for long-term use in field environments.

For automated data collection, frequency division also provides benefits. The pulses generated by a frequency-division detector are easy to interface to simple and inexpensive microprocessor circuits, which can then be employed to analyze the frequency and timing elements of the bat call in real-time. This enables less costly data recording systems, and even intelligent survey instruments, to be implemented.

It is of interest to note that many detectors that are purchased primarily for use with heterodyne and time-expansion techniques also provide a frequency-division mode. It is hard to ignore the fact that frequency division is the one mode that preserves the ability to monitor the full spectrum of bat-call frequencies in real time. This allows for the correlation of acoustic activity to observed behavior, with less chance of missing the acoustic presence of other animals that might occur with heterodyne and time-expansion modes.

To use frequency division, three features are essential. The first is a high-quality, full-spectrum transducer. Frequency division is low cost, not low quality. The use of an electrostatic microphone provides the highest quality recordings of bat echolocation calls for further analysis, whether using frequency division or any other technique. A high-quality transducer can do double duty if the detector employing it provides a buffered transducer output, which also allows full-spectrum, direct recording techniques to be employed.

The second feature required is the ability to select the division ratio. This allows the resolution of the detector to be tailored to the range of frequencies of interest. For example, if recording a low-frequency bat, such as the spotted bat (*Euderma maculatum*), better resolution is obtained by lowering the frequency-division ratio to 8:1, or even 4:1. For general purpose monitoring, 10:1 or 16:1 is the most useful. For simply monitoring and recording activity levels over time, 32:1 can be employed to maximize the density of data collected.

The remaining required feature is an adjustable sensitivity control. This adjustment facilitates setting the triggering threshold of the frequency divider to make the detector as sensitive as needed for the area surveyed, while establishing an immunity level to background noise, electrical interference, and insects. Careful use of the sensitivity control can also allow the unit to be made less sensitive to scattered bat call fragments in heavy clutter, resulting in cleaner call sequences for analysis. The ability to set the detection threshold is critical to getting the most out of frequency-division detectors.

In summary, frequency-division bat detectors provide the most robust technology available for the harsh conditions present during field monitoring and survey work. The frequency-division circuit provides the most direct and cost-effective way to collect the important frequency and timing parameters used for characterizing bat calls. Frequency division is the only detector technology affording real-time monitoring of the full spectrum of ultrasound. Pure frequency-division circuits do not retain the amplitude information required for more intricate study of bat call structure, and may not provide the most sensitive bat detection option for active surveys, but will continue to be a significant technology for acoustic bat surveys.

TIME EXPANSION: ANALYSIS CAPABILITIES AND LIMITATIONS AND FIELD DESIGN

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Time expansion is an ultrasound conversion method that retains basically all characteristics of the original signal. Hence, it is suitable for most types of signal analysis. I assess the capabilities and limitations of the time-expansion technique and suggest how to use time expansion most efficiently both in the field for immediate analysis by ear, as well as for computer analysis of transformed sounds. As an alternative to time expansion, direct high-speed recording can also be used. High-speed recording can be made e.g., using a computer equipped with a high-speed data acquisition card. I describe the differences between time expansion and high-speed recording and give an overview of material suitable for recording ultrasonic calls with time expansion or direct high-speed recording.

Key words: bat detectors, time expansion, ultrasound detectors, ultrasound playback, ultrasound recording

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INTRODUCTION

Heterodyning, frequency division and time expansion are techniques commonly used by bat detectors to transform inaudible, ultrasonic bat calls into audible sounds. The time-expansion technique is unique in the sense that it retains virtually all characteristics of the original signal (e.g., Ahlén and Pettersson 1985; Pettersson 1985,1999). The purpose of this paper is to assess the capabilities and limitations of this technique and describe as an alternative, the high-speed recording technique. Further, I describe several different time-expansion/high-speed recording systems for recording or replay of ultrasonic signals in the field.

THE TIME-EXPANSION TECHNIQUE

The time-expansion technique is similar to recording a sound with a high-speed tape recorder and then replaying it at a slower speed. However, in time-expansion bat detectors, the storage medium is digital memory rather than magnetic tape. This system has many advantages. The signal is available for replay immediately after it has been stored in the memory and it can easily be replayed over and over again if desired. Obviously, a drawback to time expansion is that it is not a real-time technique.

Perhaps the most important advantage of time-expansion detectors is that the original signal is stored in the memory. This of course means that the time-expanded signal contains the same information as the original signal, i.e., all characteristics of the original signal are retained. Thus, a time-expanded signal is suitable for any type of sound analysis. This includes spectral analysis (power spectra, spectrograms) as well as various temporal analyses (pulse length, interpulse intervals). An analysis diagram, e.g., a spectrogram, for a time-expanded signal will look just the same as that of the original signal, except for the time and frequency axes. It is, of course, relatively easy to rescale the axes to obtain the original values. Certain sound-analysis programs (e.g., BatSound, Pettersson Elektronik, Uppsala, Sweden) do this automatically.

Since time-expanded signals are stretched out in time, it is also possible for the user to hear details in the sounds that are not audible using other transformation techniques. This can be used to allow immediate identification in the field.

As noted, time expansion is not a real-time technique, so the transformed sounds will not be heard at the same time the original calls were emitted by the bat. Further, while the time-expanded signal is being replayed, it is not possible to make a new recording. In order to work efficiently with a time-expansion detector, a real-time ultrasound conversion system is also required. This allows the user to hear any ultrasonic activity in real time, and from that determine when to start and stop the time-expansion recordings. Hence, most time-expansion bat detector systems also have a heterodyne or a frequency-division system incorporated. Having a frequency-division option is especially useful as it allows for broadband detection of signals in real time.

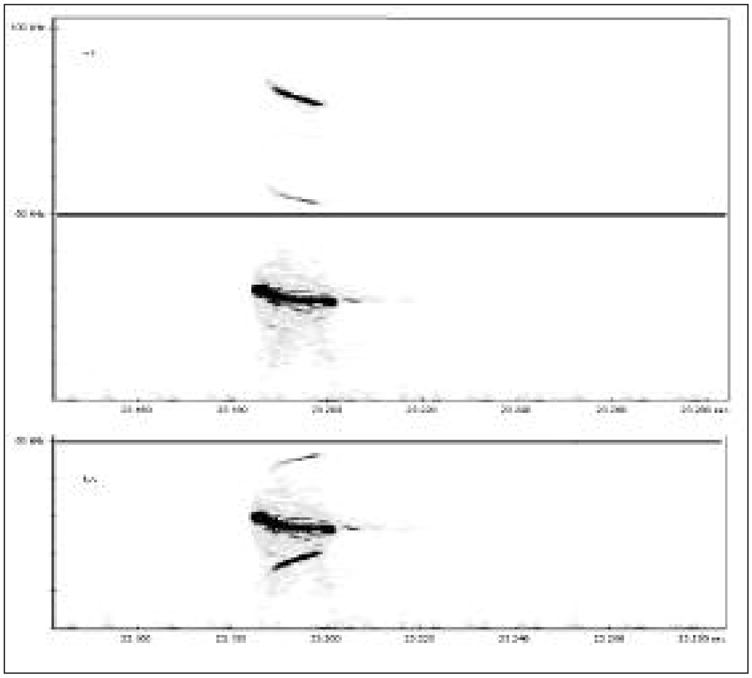
Figure 1: Illustration of aliasing. **A:** Spectrogram of the original signal prior to sampling at a sampling frequency of 100 kHz. The Nyquist frequency is indicated. **B:** Resulting, aliased signal after sampling.

Drawbacks to the time-expansion technique are the limited storage time, typically from about one up to a few tens of seconds, and the somewhat higher cost.

TIME-EXPANSION DETECTORS IN PRACTICE

When time-expansion bat detectors are used in the field, several things need to be considered. First, the time-expansion technique transforms the full frequency range, i.e., it is a broadband technique. However, there is always an upper-frequency limit above which time expansion is unable to record signals correctly. Since the time-expansion system uses digital memory, the original, analog signal first has to be sampled and converted into a digital signal. This is accomplished by an analog-to-digital (A/D) converter. The number of samples taken per second is called the sampling rate or sampling frequency and is measured in Hz or Sa/s (samples per second).

The so-called sampling theorem states that the signal should be sampled with a sampling frequency of at least twice the highest signal frequency (or Nyquist frequency). If this rule is not followed, aliasing occurs. This means that signal frequencies above half the sampling frequency are folded over to lower frequencies (Fig. 1).

Applying a low-pass filter with a cutoff frequency less than half the sampling frequency prior to sampling the signal eliminates, or at least attenuates, the frequencies that would otherwise be aliased. In order to completely eliminate these frequency components, a very sharp cutoff filter is required. In practice, this anti-aliasing filter is often not sharp enough to completely remove all frequencies above half the sampling frequency, so it is important to learn how to recognize aliased frequency components in a spectrogram.

When the signal is converted into digital form, it must also be quantized, i.e., each sample is assigned one of a limited number of digital levels (values). The available number of digital values depends on how many bits are used for the quantization. As an example, if 16 bits are used, then there are $2^{16} = 65{,}536$ levels. Obviously, quantization becomes more accurate with a larger number of levels. The quantization process adds a certain amount of noise, depending on the number of bits used, with more noise resulting if fewer bits are used. From a qualitative point of view, it is thus better to use as many bits as possible. However, there is usually a certain amount of noise present in the signal even before it is digitized. The number of bits used for the quantization does not affect this noise.

Since time-expanded signals are often used for different types of signal analysis, it is particularly important that measures are taken to ensure high sound quality. The gain of the detector's input amplifier should be adjusted to avoid overload of the amplifier. An overloaded amplifier generates spurious harmonics, making it impossible to accurately interpret the spectrogram or power spectrum of the signal. All amplifiers add a small number of harmonics to the amplified signal even at levels below clipping (overload). This distortion is usually small, but increases dramatically as the signal level reaches the clipping level. A clipped waveform can be detected by studying the peaks of the signal in the oscillogram. As the clipping level is reached, the peaks become rounded off or clipped.

It is important that the microphone used has a reasonably flat frequency response and a high signal-to-noise ratio.

A time-expansion detector requires some mechanism to start and stop the recording process. In the manual triggering mode, a button is used to start and stop recording. This gives the user full control over the recording, but also requires manual operation of the detector. In the automatic triggering mode, the detector is normally recording continuously. When a sufficiently strong signal is detected by the triggering circuitry of the detector, the recording is interrupted after a certain time. The recording parameters can be adjusted to ensure that the time-expansion memory contains a recording of the signal from a certain time prior to the triggering instant to a time after the triggering instant. This avoids cutting off the beginning of the first pulse. Furthermore, the triggering source can be either the entire signal frequency range or a narrowband signal. The former makes the time-expansion system sensitive to all ultrasonic signals, while the latter will make the detector react only to signals within a certain frequency band. This can be used to avoid triggering by undesired signals such as low-frequency noise.

The fact that time-expansion detectors store the original signal in the memory allows for other important applications. It is possible in some detectors to replay the signals at the original speed, rather than a lower speed that is used under normal circumstances. If a suitable ultrasonic loudspeaker is available, the time-expansion detector can be used for playback experiments of ultrasonic signals. When the signal is replayed at its original speed, it can also be fed to the heterodyne input of the detector, if available. The resulting signal from the heterodyne system will be exactly the same as that recorded in real time originally, enabling the user to carefully listen to the heterodyned signal over and over again. This is of value for immediate identification of bats in the field. Both of these features are available in some bat detectors, e.g., the Pettersson Elektronik D240X.

HIGH-SPEED RECORDING

With a time-expansion bat detector, it is also possible to make recordings of the original signal, albeit in a slowed-down version. As mentioned, the limited memory size constrains the maximum recording time. Although the recording time feasible with time-expansion bat detectors is usually sufficient for recording bat calls, there are situations when longer recordings are required. In such cases, high-speed recording of the original signal can be an alternative to the time-expansion technique.

High-speed recording can be done in a number of different ways, for example, using a computer with a high-speed data acquisition card. Other methods include using a high-speed digital audio tape recorder or instrumentation tape recorder, a device supporting the DVD audio format or a specialized high-speed recording device, using a hard drive or a flash memory card as storage medium.

Recording ultrasonic signals directly requires a fairly large memory size. Sampling at 300 kHz with a resolution of 8 bits requires about 18 MB of memory per minute or 1 GB per hour. However, with the size of today's hard drives, storing large files should not be an issue, and it is possible to obtain many hours of recording time.

In addition to the increased recording time, the high-speed recording technique also means higher sound quality than most time-expansion bat detectors, since the latter usually incorporates both an A/D conversion and a D/A conversion. In order to analyze the signal with a computer, a second A/D conversion is made. In most high-speed recording devices, only one A/D conversion is needed, which is obviously an advantage.

Since high-speed recording is simply a recording technique and not an ultrasound transformation technique, ultrasonic calls are not made audible, at least not in real time. Consequently, it is often practical to also use a simple, real-time transformation bat detector such as a heterodyne or frequency-division model. This will allow the user to obtain information about ultrasonic activity in real time and thus determine when to start and stop the recordings.

Although a high-speed recording device can be made relatively small, a time-expansion bat detector with an audio cassette or digital audio tape recorder is usually a less expensive solution, so the former should not be considered a direct substitute for a time-expansion detector.

EXAMPLES OF SYSTEMS FOR TIME-EXPANSION AND HIGH-SPEED RECORDING AND PLAYBACK

There are a variety of systems for recording and playing back signals with time-expansion or high-speed techniques. Factors to consider when purchasing such a system include the degree of ruggedness for field work, the sound quality, and the maximum recording time.

Time-Expansion Bat Detector and a Tape Recorder

A time-expansion bat detector and a tape recorder (regular or a digital audio tape recorder) usually constitute a highly portable, rugged, and inexpensive solution. For optimum sound quality, the tape recorder should have a manual recording level control. In the case of a regular cassette tape recorder, high-quality cassette tapes, such as CrO_2 or metal types, should be used.

For automated, unattended recording of bat calls, a self-triggering bat detector is required. In order to have the tape recorder make recordings automatically, a voice-activated tape recorder can be used. Some time-expansion bat detectors also have a tape recorder control output that can be used to make the tape recorder start and stop automatically. The total recording time in the automatic recording mode is obviously limited by the recording time of the cassette.

When a simple cassette tape recorder is used, one should consider non-ideal performance, e.g., distortion, speed variations, and limited high-frequency response. Furthermore, cassette tapes are not ideal for the long-term storage of data.

Time-Expansion Bat Detector and a Computer

Instead of using a tape recorder to record the transformed sounds, a laptop computer with suitable software can be used. This combination provides relatively high sound quality, although it is somewhat less durable for work in the field.

In order to automatically make recordings using this system, the program used for recording must have a recording mode that starts recording above a certain sound level and stops below this threshold. The "BatSound" software (Pettersson Elektronik, Uppsala, Sweden) has such a mode, in which a time stamp is also saved in the recorded file to facilitate recovering the actual date and time the recording was made. Using this system, total recording time is limited by hard-disk space, so recording several hours of sound is feasible.

Ultrasound Microphone and a Computer with a High-Speed Sound Card

Provided the computer is equipped with a data acquisition card supporting sufficiently high sampling rates (typically > 300 kHz, preferably a 500 kHz sampling rate), the ultrasonic signal can be fed directly into the computer without first being transformed by a bat detector. Software that supports the high-speed card is also required. BatSound Pro (Pettersson Elektronik, Uppsala, Sweden) supports a number of high-speed cards and is capable of automatically starting a recording when the sound level exceeds a certain threshold.

As described above, a bat detector is often a practical means to complement this recording method. In that case, the amplified signal from the microphone of the bat detector can be used for the high-speed recording. This system makes for the highest sound quality and is capable of generating long, continuous recordings. It is, however, not as rugged and easy to use as the bat detector/tape recorder system.

Replay of Ultrasonic Signals

Signals obtained using time-expansion or direct high-speed recording can be used to replay the original ultrasonic signal. As described above, some time-expansion bat detectors allow replay of the stored signal at the original speed. A more versatile ultrasound replay system is obtained by using a laptop computer with a suitable program (e.g., BatSound Pro) and a high-speed sound card (e.g., DAQCard 6062E, National Instruments, Austin, Texas) which has both A/D and D/A converters and an ultrasound loudspeaker.

This is a convenient system because it allows the user to first make recordings with the high-speed card, then uses software (e.g., BatSound Pro) to edit these and finally compile a play list for high-speed replay. Sampling frequencies of up to 500 kHz are feasible.

In exceptional circumstances, researchers may need to record ultrasound on several channels simultaneously (e.g., for acoustic localization procedures). In the field, this can be achieved by using multi-channel instrumentation recorders, by specialized high-speed multi-channel data acquisition cards (e.g., some used with the software Avisoft (Avisoft, Berlin, Germany)), or with a new generation of data acquisition recorders, such as the DiSC6 (Heim Systems, Bergisch Gladbach, Germany), which samples at 1280 kHz spread over 6 channels and can record to Advanced Intelligent Tape.

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ZERO-CROSSINGS ANALYSIS FOR BAT IDENTIFICATION: AN OVERVIEW

CHRIS CORBEN

In this paper, I present an overview of the effectiveness of Zero-crossings Analysis (ZCA) for the identification of free-flying bats, based on 17 years of experience using the Anabat system. My opinions are based on intimate involvement with ZCA, as the original designer of the hardware and software used by Anabat, and also as one who routinely uses Anabat as a major component of field surveys for bats. I explain how the system works and how it compares with other approaches to the general problem of making echolocation calls available to humans for bat identification. I argue that ZCA has numerous advantages over other approaches, especially for passive monitoring. I also offer my views on a number of general issues pertaining to the use of acoustic monitoring for bats.

Key words: Anabat, bats, echolocation, Fast Fourier Transform, frequency division, heterodyne, spectral analysis, zero-crossings
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INTRODUCTION

I became involved with bat acoustics in the mid-1980s when I saw the chance to combine my passions for electronics and wildlife in the design of a practical bat-detection system. I was intrigued by papers about the use of bat echolocation calls for species identification, but I found that equipment then available for making use of ultrasonic signals was too expensive for my budget. I realized that recent advances in computer hardware would make it possible to find other solutions. Consequently, I resolved to develop a less-expensive system, better tailored to the specific needs of bat acoustic identification. I designed both hardware and software for a system based on Zero-Crossings Analysis (ZCA), subsequently developed as a commercial product called Anabat (Titley Electronics, Australia, *www.titley.com.au*). In this paper, I use the terms Anabat and ZCA interchangeably because Anabat is the implementation of ZCA with which I am most familiar, and which is most widely used for monitoring bats, at least in Australia and North America.

In recent years, my main employment has been to survey bats for various purposes, and to this end I have made extensive use of ZCA, as implemented by Anabat. I find this system to be effective for both field identification of bats and passive monitoring of bat activity. Using Anabat has allowed me to treat bats as field-observable animals, in much the same way as birds or frogs.

For the symposium, I was originally asked to write on the subject of frequency division, but this is a relatively minor part of the whole system, so I mostly address ZCA and its strengths. To do so requires comparing ZCA with spectral analysis, which is the principal alternative means of visually displaying bat calls. I argue that ZCA is a more practical and effective approach to echolocation-call analysis for species-identification purposes. I have extensive experience with spectral analysis, mainly for analyzing frog calls, though I have also used it to analyze vocalizations of birds and bats, and other sounds. I greatly admire the concept of spectral analysis, and I think of the Fast Fourier Transform (FFT) as one of the most elegant mathematical inventions. The sounds of most animals are best analyzed using spectral analysis, but for identifying free-flying bats, this approach is technological overkill because bat echolocation calls are simple signals well suited to analysis by ZCA. It is therefore appropriate to make use of the many advantages ZCA offers in cost and efficiency. In my view, spectral analysis does not provide clear advantages that offset its practical liabilities for this purpose.

HOW DO FREQUENCY DIVISION AND ZCA WORK?

One way to determine the frequency of a signal is to count how many cycles, or vibrations, occur in a given time interval. This works well when dealing with continuous signals of constant frequency. However, bat echolocation call frequencies typically change rapidly, so another approach is required. Instead of counting cycles, the duration of each individual cycle can be measured and the frequency computed from its reciprocal to produce a graph of frequency against time. This is the basis of ZCA.

In practice, it is often advantageous to first derive a second signal, which produces one cycle for each group of several cycles from the original signal. This is the process of frequency division. Using it has the advantage that the frequency-divided signal is downshifted in frequency by a constant factor, the division ratio, making it audible to humans. Another advantage is that a frequency-divided signal averages out small fluctuations, giving a smoother representation, better suited to the level of detail needed to see the underlying structure of a bat call. A lower division ratio displays more points in the frequency display but at the cost of increased storage requirements and processing power, and if the extra detail is not helpful for species identification, then it is superfluous. An excessive division ratio results in too much averaging and insufficient detail in the display. The optimum division ratio for practical use is a matter of compromise between these competing needs. I use a division ratio of 16 for most purposes, although 8 is a better choice for displaying very steep calls of short duration, especially at low frequency.

Zero-crossings Analysis ignores the intensity of the original signal and only responds to the highest intensity frequency component at any instant. These limitations have little, and in many cases no, practical influence on the usefulness of ZCA for bat-call identification. One consequence, however, is that a ZCA-based system is inherently less sensitive than a system based on spectral analysis, since ZCA lacks the ability to separate simultaneous signals of different frequencies.

USE OF ZCA IN ACTIVE MONITORING

With active monitoring, a visual display of bat-call frequency characteristics is generated in real time by feeding the output of an Anabat detector into a laptop computer through an interfacing device called a ZCAIM. The Anabat software running on the computer draws the display on the screen and saves data into files stored on the hard disk for later retrieval and more detailed analysis if required.

When I undertake active monitoring, I try to observe any bats detected acoustically by illuminating them with a spotlight. This allows me to see features that aid identification. Such features include color, wing shape, flight pattern and feeding behavior, and some other distinctive characters such as the long ears or tails of certain species. The combination of visual observations with call features displayed on the screen allows the greatest likelihood of identifying free-flying bats.

I find that some groups of bat species are hard to distinguish acoustically but easy to identify visually. Examples include *Lasionycteris noctivagans*, *Eptesicus fuscus* and *Tadarida brasiliensis* in North America, and *Vespadelus darlingtoni* versus *Miniopterus schreibersii*, *Scotorepens orion* versus *Scoteanax ruepellii* and *Mormopterus beccarrii* versus *Saccolaimus flaviventris* in Australia. Many species look very similar in the field but are easily identified acoustically, examples being *Myotis yumanensis* versus *M. lucifugus* and *Nyctinomops macrotis* versus *N. femorosaccus* in North America, and *Vespadelus darlingtoni* versus *V. pumilus*, *Scotorepens orion* versus *S. greyii*, and species of the families *Hipposideridae* and *Rhinolophidae* in Australia. Thus, I find acoustic and visual cues to be complementary, and the combination of both is typically much more powerful than either by itself. (See Limpens this volume.)

Active monitoring is also an excellent tool for learning how a bat's calls change with the varying circumstances under which it flies. A real-time display, such as that offered by Anabat, in combination with visual observations, provides an observer with an immediate link between the behavior or circumstances of a bat and the types of calls it produces. This link enhances comprehension of a species' acoustic repertoire. Understanding such variation is essential to developing effective identification criteria.

With practice, it is quite easy to visually observe bats and to make use of information on the computer screen at virtually the same time. The most difficult part of the process is often locating a bat with the spotlight, especially because it is usually undesirable to illuminate the bat before it has made its closest approach. Most bats are repelled by the light, typically turning away from it and consequently changing their calls and also reducing the likelihood of the observer obtaining adequate views. Actually seeing the necessary features of a flying bat is not easy and requires practice. Much of the skill required involves understanding the kinds of views that are most informative, and developing the ability to make the best use of the brief moments when a bat is in the best position for viewing. This is analogous to the problem of observing critical identification features on many birds, such as distant birds of prey or seabirds.

USE OF ZCA IN PASSIVE RECORDING

With passive recording, a detector and storage system are deployed so as to record bats in the absence of any human observer. Using Anabat, such systems have typically been implemented using a detector, ZCAIM and laptop computer, though tape-recorders have often been used for storage.

To make passive recording easier, I designed a device called a Storage ZCAIM, which replaces the ZCAIM/computer combination with a single, small, low-power device that stores all recorded data on a Compact Flash card. It can be powered for several nights using AA batteries, and the data can be downloaded from the Compact Flash card as Anabat files ready to view.

Passive-recording systems can be deployed near the ground, but can also be raised into the tree canopy or suspended from a balloon. Passive recording offers many options not open to active monitoring. Using ZCA, many passive-recording systems can be deployed by a single operator, providing insight into spatial heterogeneity not otherwise available. Passive recording can also be conducted in places difficult for active monitoring, for example, to avoid disturbing local landowners. Furthermore, passive recording largely overcomes the problem of temporal variation, since it is easy to collect data all night or for many consecutive nights. Long-term passive-recording stations using ZCA are now in use, allowing bats to be recorded all night every night, year after year.

Passive recording is less effective for species identification than active monitoring, mainly because visual cues are lacking. Passive monitoring also records bats at a lower rate. In experiments I conducted, active monitoring recorded 3 times as many bats and 3 times as many calls per bat as passive recording over the same time period. This results from the ability of an active observer to continually reorient the microphone, increasing the chance of detecting a bat irrespective of its direction. An active observer can also keep the detector oriented towards the bat after detection, thus obtaining longer sequences, often of better quality.

ADVANTAGES OF ZCA

Comparisons with Spectral Analysis

Most of the advantages of ZCA over spectral analysis come from three unavoidable realities: ZCA requires less storage space, is faster, and demands less power.

Storage space. To make effective use of spectral analysis, it is necessary to make recordings of bat calls using a high sampling rate, typically over 300,000 samples per second at 12 bits per sample. Such a sampling rate is still inadequate for the highest frequencies used by bats. Furthermore, only 12 bits of resolution gives a poor dynamic range, making it difficult to avoid overloading. In contrast, ZCA only requires storage of the intervals between zero crossings in the frequency-divided signal, and these are only stored when bats are present and overloading is impossible. A full-spectrum recording of a single bat call typically requires at least 100 times more storage space than ZCA, but only if compressed in a way which compromises the sensitivity advantages of spectral analysis. In practice, recordings for spectral analysis typically require at least 3 orders of magnitude more storage space than ZCA.

Analysis processing speed. The speed difference arises because of the large amount of data that must be processed for spectral analysis, the computational demands of the FFT, and the graphical processing required to draw the display. In contrast, ZCA works with tiny datasets and uses simple calculations to draw simple graphics. The difference is hard to quantify, because so many variables influence the speeds of these processes. In my experience, as one who regularly uses Anabat to quickly scan through large numbers of bat-call files, I find the equivalent task using spectral analysis to be so slow that it is often impractical.

Power consumption. The power consumption differences between ZCA and the devices necessary for spectral analysis have implications at every level, but especially for deploying multiple recording units in the field. It is currently feasible to deploy a time-expansion system for passive recording using relatively low-power devices, but this approach sacrifices the ability to monitor continuously for extended periods. To achieve continuous monitoring for spectral analysis would require use of a high-powered laptop at each monitoring site and some way to power it. By contrast, a long-term recording station using ZCA and a storage ZCAIM could run for a week on a single 12V 7AH rechargeable battery, and a small solar array could keep the battery charged.

These performance differences between spectral analysis and ZCA are most evident in passive recording, for which the low power requirements and low storage overhead make the combination of frequency division and ZCA ideal. In my opinion, the resources of power, money, and personnel required to implement a single recording station for spectral analysis would be better used to operate several stations based on ZCA.

ZCA versus Auditory Methods

Visual display of bat calls is not the only way to use acoustics for species identification. Ahlén and Baagøe (1999) advocate the use of a combination of visual observations with acoustic input from bat calls rendered audible by both heterodyne and time-expansion down-conversion. They emphasize the importance of auditory observation and relegate visual display of call characteristics to a minor role. From my own experience, I agree with their observations on how to most effectively combine visual and acoustic cues to identify free-flying bats, and the importance of experience and a knowledge of the biology of the bats to successful field identification. However, I see no reason to assume that this approach will be superior to the use of frequency-divided sound and a real-time display as offered by Anabat.

Purely auditory observations suffer from some inherent problems. A heterodyne system provides a sufficiently competent observer with information about the nature of the frequency/time course of a signal, but only if the observer listens to the same bat over an extended period while adjusting the frequency to which the detector is tuned. In contrast, ZCA enables a real-time display in which the most important characteristics of a single call are immediately apparent.

Time expansion offers more auditory information from a given bat call by stretching it into a time scale better suited to our neural processing, but this comes at the cost of limiting the recording time available in a particular setting. In my experience, it is very common to encounter bats in mixed flocks, where several species move past a recording site in a matter of seconds, often after a long break in activity. Recording of such events is not currently feasible using time expansion, because of the long time period required to play back the time-expanded signal before another can be recorded.

Currently, ZCA requires the use of a laptop computer in order to offer real-time displays, but this will change since the technology to produce such displays in a highly portable form already exists. When handheld real-time displays become readily available, I expect that much of the value of auditory observation will be supplanted by visual displays. Even though Ahlén and Baagøe (1999) found complex acoustic cues revealed by time expansion to be important to field identification of bats, this does not preclude the possibility that ZCA-based real-time displays will perform just as well or better. People inevitably favor the technologies with which they are most familiar. In my experience, real-time visual displays of bat calls play a pivotal role in species identification, providing the immediate feedback between visual and acoustic cues, which Ahlén and Baagøe value. Additionally, providing a visual display of bat calls will benefit the many people who Ahlén and Baagøe regard as having a "lack of 'ear.'" In my experience, observers working with other groups of sound-emitting animals, such as birds or frogs, typically find it easier to make identifications by sight than by ear, and relatively few people are really effective at using their hearing.

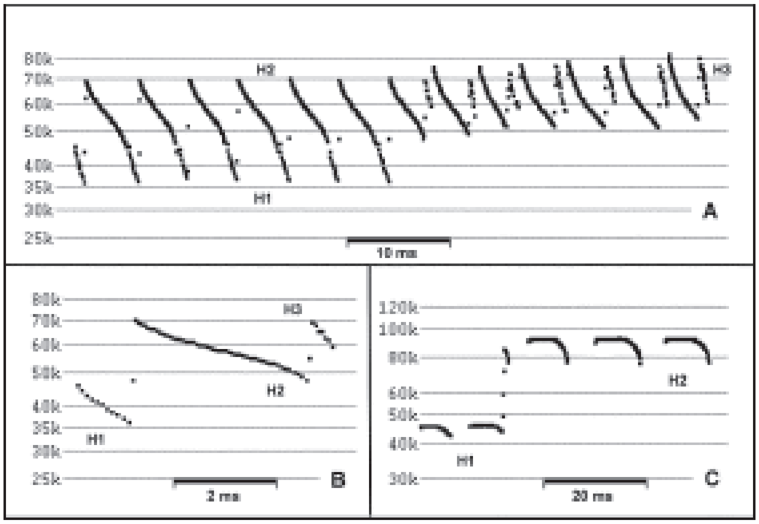

Figure 1: Display of harmonics by Anabat. **A:** Consecutive calls of *Macrotus californicus* recorded at Sand Tank Mine, Arizona, showing 1st, 2nd, and 3rd harmonics (H1 to H3) as displayed in compressed mode by Anabat. **B:** The middle call from sequence in A, showing harmonic switching from H1 to H2 and then H2 to H3. Single dots between harmonics show cases where intermediate frequencies have been displayed. **C:** Five consecutive calls of *Rhynchonycteris naso* recorded at Chan Chich Creek, Belize. Time intervals between calls in A and C have been removed or reduced to show adequate detail of several calls in one graph.

DISADVANTAGES OF ZCA

There are two aspects of a ZCA display which fall short of what can be produced using spectral analysis: the handling of harmonics and the amplitude envelope.

Harmonics. ZCA can only respond to one signal at a time. If more than one signal is present at any instant, ZCA will always respond to the signal with the highest intensity. One consequence of this is that ZCA can only respond to one harmonic at a time.

Bat echolocation calls have a simple structure compared to most bio-acoustic signals, with most of their energy typically radiated in 1 to 3 harmonics. Using ZCA, most bats are consistently detected on just a single harmonic, but many bats are detected on 2 or more. Because of the way ZCA works, a harmonic must become dominant before it can be detected. To become dominant, a harmonic must be of greater intensity than any other harmonic, from the point of view of the detector. The intensity with which a detector perceives a signal depends on the intensity at which the bat emits the sound, the degree to which the signal is attenuated by the atmosphere (which depends on the weather), and upon the sensitivity and frequency response of the detector itself. Thus, a number of factors interact to determine which harmonic is dominant, unless the energy radiated in one harmonic is sufficient to ensure its dominance.

ZCA can reveal harmonic structure in many situations because of its ability to display many calls quickly. While it is not possible to simultaneously view more than one harmonic, the harmonic structure can often be inferred because of an interaction between the frequency responses of the air and the bat detector. This interaction results in different harmonics becoming dominant at different times as the distance between bat and detector changes.

Figs. 1a and 1b show Anabat displays of harmonics in the Phyllostomide bat *Macrotus californicus*. In Fig. 1a, depicting an actual Anabat sequence, three different harmonics can be seen. While the 2nd harmonic (H2) was always dominant for most of each call, the first harmonic (H1) was detected for the early part of each call when the bat was at a distance, and the 3rd harmonic (H3) was detected late in each call when the bat was closer. One call, depicted by itself in Fig. 1b, shows all 3 harmonics. Note that the switch between H1 and H2 in the first 7 calls always occurred when H1 reached 35 kHz, despite the fact that the distance between the bat and the observer must have been changing. This indicates that the bat itself was largely controlling this harmonic switching by shifting energy from H1 to H2 at that point in each call. While the frequency at which the switch between H2 and H3 occurred varied somewhat between calls, it always occurred at a point where the slope of the call changed abruptly, suggesting that the bat was largely in control of that harmonic switching also.

Spectral analysis is a much better tool for revealing the underlying harmonic structure of a signal, and it should always be used if understanding harmonic structure is the main objective. However, ZCA is surprisingly effective at revealing the harmonic structure in those species for which the harmonics are of most importance for identification.

While spectral analysis provides more harmonic detail than ZCA, the extra detail has not, to the best of my knowledge, been shown to be critical to bat call identification. Ironically, those who argue that Anabat is "confused" by harmonics typically draw examples from species whose harmonic structure is revealed by Anabat in sufficient detail for identification of the species concerned. An example is *Rhynchonycteris naso*, for which Fenton et al. (1999) claim Anabat "may not be reliable," citing their own detection of its calls using Anabat as at 98-100 kHz, while O'Farrell and Miller (1997) reported detecting it at 47 kHz. In fact, *R. naso* emits both a 1st harmonic at 45 to 50 kHz and a 2nd harmonic at 90 to 100 kHz, and either or both can be detected by Anabat, depending on circumstances. On either harmonic, *R. naso* calls are distinctive enough to be recognized without any difficulty (B. Miller pers. comm.; pers. obs.). The harmonic structure is revealed by Anabat's ability to detect either harmonic, but this has no bearing on how reliably the call can be identified. Fig. 1c shows consecutive calls from a single *R. naso* recorded at Chan Chich Creek, Belize. The 1st call is typical of those recorded while the bat is relatively distant, while the later 3 calls are typical of those recorded when the bat is closer to the observer. Note the transition from dominance of H1 to H2, and the switch between harmonics during the 2nd call.

In a number of cases, such as *Corynorhinus townsendii* and *Macrotus californicus*, the ability of Anabat to detect more than 1 harmonic is an important feature aiding identification of those species in the field. Parsons et al. (2000) reported that in cases such as these, ZCA can result in a signal jumping between harmonics, "leading to a misleading output signal." In fact, there is nothing misleading about the output; the 2 or more detected harmonics are readily interpreted as such and the harmonic structure is revealed by Anabat in a useful way (see Fig. 1). While some dots appear at intermediate frequencies during the switch from 1 harmonic to another, these should not mislead an informed user.

Anabat could not be used to discriminate between species that differ only by features of a harmonic, which never becomes dominant. This is a very specific requirement, where 2 bat species have calls identical in the dominant harmonic, but differing in features of some other harmonic. Since frequencies of harmonics are fixed in relation to the fundamental, this means that the specific differences between bats would have to be in the relative intensities of harmonics. I am not aware of any such cases.

Amplitude. ZCA does not give any direct information about the amplitude envelope of a bat call, though some information can be deduced from observing how call features appear to change as the bat-to-detector distance varies. Spectral analysis, on the other hand, provides several different views of a call and can reveal how its amplitude varies in time.

It is a widely held view that there might be something useful for species identification in the amplitude envelope, and I am often asked why I didn't incorporate this ability into Anabat. The answer is that I designed Anabat to be as fast and efficient as possible and incorporating amplitude information would have compromised this goal. So far, I have seen no evidence that retention of amplitude information is necessary for species identification.

In their efforts to quantify bat-call characteristics, researchers have used a number of parameters which depend on the amplitude envelope. The type of parameter most frequently used is some version of the frequency of peak energy (Fp), that is, the frequency at which the call contains the most energy. Such a parameter has been found important for discriminating between species (e.g., Parsons and Jones 2000). Since the amplitude envelope is essentially inaccessible to ZCA, it follows that such parameters are not available to Anabat users. However, the fact that a parameter has proven useful does not mean it is essential.

The most easily measured frequency parameters, maximum frequency (F_{max}) and minimum frequency (F_{min}), are affected by recording conditions, because they often occur at the start or end of a call, when the amplitude is at a minimum and changing rapidly. Therefore, measurements of F_{min} and F_{max} can be profoundly influenced by the distance and orientation of the bat. Thus we have a need for other, more effective, frequency parameters.

This problem will be familiar to anyone trying to communicate bat-call identification criteria. How do you characterize the frequency of a bat call with a single figure when the call covers a wide range of frequencies? F_{max} would be a bad choice for most calls, although it would seem the best choice for a species of *Rhinolophus*. F_{min} would be a poor choice for many call types, but would seem the most appropriate for calls which are "J" shaped, such as produced by species of *Vespadelus*. A call could be characterized operationally by choosing the frequency to which a heterodyne type bat detector responds to it in a particular way. Alternatively, Fp could be used if spectral analysis is being employed. It would be better, though, to find a parameter that is meaningful to anyone working with bat calls.

A common factor in the frequencies that seem to best characterize bat calls is that they correspond to the portions of calls where the frequency-time curve is "flattest", i.e., where the absolute slope of the call is at a minimum. In the late 1980s, I built into Anabat the facility to automatically estimate a parameter I termed Characteristic Frequency (Fc), which is the frequency at the end (along the time scale) of the flattest part of a bat call. For calls with an extended linear portion, where the flattest part covers a range of frequencies, other choices could have been made, but taking the frequency at the end of that section seemed the most parsimonious, since it allows the closest match between calls that seem very similar but differ in the degree of curvature.

My contention, then, has been that while Fp might be a useful parameter, Fc could work just as well for distinguishing between species, if it was calculated instead of Fp. This contention remains to be tested, but if true, it would mean that Anabat's inability to measure Fp would be offset by its ability to measure Fc. Fc is also available to those using spectral analysis, though it is less easy to compute than Fp without imposing some subjectivity on just where it is measured. In the absence of tests comparing Fc with Fp, some insight can be gained from looking at just what Fp means and how it might relate to parameters available to Anabat users.

It is important to realize that Fp can be measured in many different ways, and the method used will have an influence on the value obtained. Most publications making use of Fp, in my opinion, provide too little detail in the methods section to allow another user to duplicate the approach. Most users have measured Fp from a power spectrum, which is a graph of the energy content within each frequency band, calculated using the FFT and summed over a whole bat call, or even several calls. However, there are still many ways this could be done. Perhaps the best explanation of how to approach this was provided by Parsons and Jones (2000), who used a single FFT slice to cover a whole call. However, they erred in using a Hamming windowing function in this process when they should have performed it without a windowing function (often described as using a rectangular window). Their use of a windowing function inevitably imposes a peak on the power spectrum even if one didn't actually exist, and furthermore, the position of the peak along the frequency axis could be influenced by the position of the call within the FFT slice.

Another approach which would result in a different value for Fp would be to determine at what point in a call the amplitude envelope reached its maximum value, and determine the frequency of the call at that point. This approach assesses the maximum energy in the call at any one instant, rather than integrated over the whole call. A very important distinction between the instantaneous value and the integrated value is that the instantaneous value is affected only by the amplitude of the signal, whereas the integrated value (from the power spectrum) is affected by both the amplitude and the slope of the signal, since the energy in a particular frequency band is controlled in part by the amount of time spent in that band.

Fp could also be measured by determining the frequency at the point in a spectrogram where the amplitude variable reaches its peak. The darkest coloring in a spectrogram would typically indicate this, or the highest point in a waterfall or landscape diagram. See Ahlén (1981) for examples of these types of display. If measured in this way, the value of Fp would depend on both amplitude and slope, but the degree of dependence on slope would be affected by the size of the FFT slices and the windowing function used in generating the display.

My own investigations into the use of Fp suggest that the amplitude envelopes of recorded bat calls tend to be variable, even within one individual bat. This could partly result from artifacts of the recording process, for example, how well the microphone is kept oriented towards the bat being recorded. This factor can have a profound effect, because the directionality of a microphone depends on signal frequency. Conversely, the slope characteristics of bat calls show much more consistency, and of course, are closely tied to the frequency-time course or "shape" of a call.

Bullen and McKenzie (2002) used another approach to measuring Fp by using the FFT to generate power spectra from frequency-divided calls, even with Anabat detectors, which make no attempt to preserve the amplitude envelope. The interesting aspect of their approach is that it produced power spectra in the absence of any amplitude information. This induced me to program into Analook the facility to produce power spectra of calls analyzed using ZCA. The resulting frequency parameter (Fpz) is an analog of Fp, but dependent only on call slope. It is calculated using a computationally much simpler and faster approach than the FFT and effectively provides the frequency at which the call spends the most time.

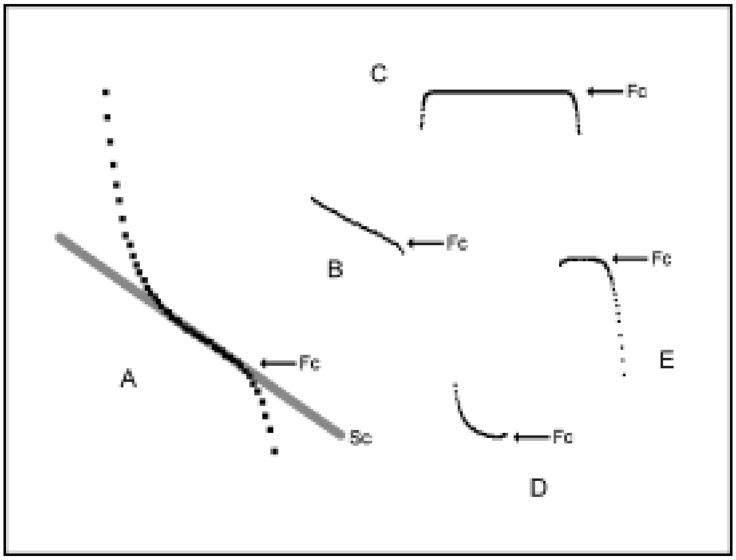

Figure 2: Measurement of characteristic frequency (Fc) and characteristic slope (Sc). **A:** *Myotis yumanensis*. The gray line labeled Sc indicates the slope of the flattest part of the call. **B:** *Tadarida brasiliensis*. **C:** *Rhinolophus megaphyllus*. **D:** *Vespadelus darlingtoni*. **E:** *Noctilio leporinus*. Calls are not to scale.

It remains to be determined how Fc, Fpz and the various forms of Fp compare with each other under different conditions. However, I think that any of these parameters would, by itself, provide powerful discriminating ability between bat species. Furthermore, any of these parameters could effectively be used as an indicator frequency, that is, the single measure that would most effectively communicate to another interested person the important features of a bat call. So I doubt that the inability of Anabat to access amplitude dependent parameters such as Fp will be of much, if any, consequence to species identification.

Comparisons of Different Approaches

It is not easy to compare the effectiveness of different systems for the ultimate goal of identifying bats. One reason for this difficulty is that any worker who is familiar with a particular system will tend to find a rival system inferior, because it produces an unfamiliar output. Thus people used to spectral analysis tend to find the Anabat style of output too simple and grainy, as though it is a poor representation of a much more complex whole. On the other hand, since I am used to looking at Anabat screens, what I see in an FFT-based spectrogram is blurry and imprecise, making it difficult for me to identify familiar bats. At another level, I find the use of spectral analysis frustratingly slow.

Some insight into the effectiveness of different systems can come from the overall perceptions of those most familiar with them. A recurring theme from those

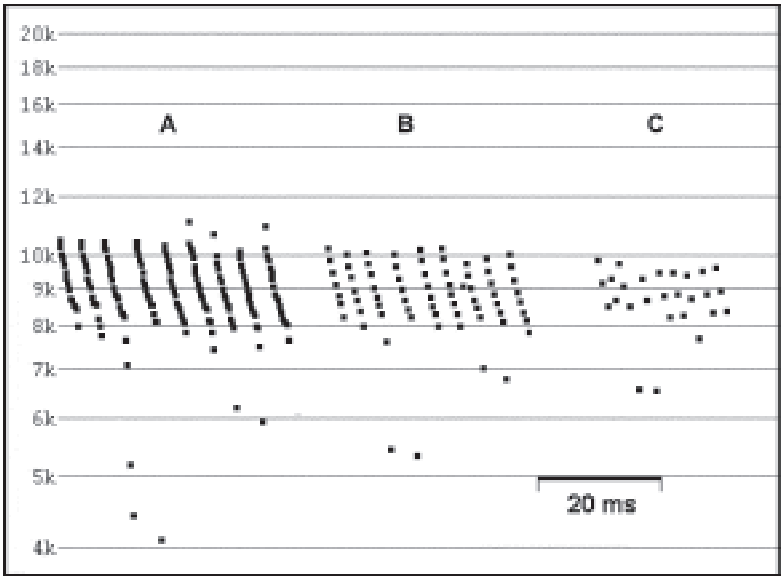

Figure 3: Effect of division ratio. The same sequence of 9 consecutive calls of *Euderma maculatum* as displayed at division ratios of **A:** 4, **B:** 8, and **C:** 16. Note that even at a division ratio of 16, these calls could be easily identified as *Euderma*, although they may be difficult to see among low-frequency noise. Many displays of *Euderma* calls have more dots than these. Note that a division ratio of 4 in general should not be used, since frequencies higher than about 40 kHz will be aliased down to lower values and could be misleading. The extra detail afforded by using 4 over 8 would not be of any consequence to recognizing the species.

who use acoustics for species identification is that some species are easy to identify, some are identifiable under favorable conditions, and some are hard to distinguish. This accords with my own experience using Anabat. Furthermore, reports about the problems experienced by other workers are similar to the problems I encounter myself. For example, the comments by Ahlén and Baagøe (2000) on "other *Myotis* species" sound very close to my own experiences with certain groups of *Myotis* in North America. Furthermore, the general tone of the particular identification issues discussed by Ahlén and Baagøe sounds very close to my own impressions of identifying bats using Anabat in North America or Australia. I therefore suggest that for each of the methods – ZCA, spectral analysis and auditory observation – the problems of species identification are similar. Each approach has its strengths and weaknesses, and to its own adherents, each is superior.

SPECIFIC CRITICISMS OF ANABAT

Over the years, Anabat has faced a number of criticisms, many of which I feel are unjustified. These often show a lack of familiarity with the system, or are based on prejudices over what a bat-call analysis system should provide. I discuss a range of these common criticisms below.

Anabat isn't as sensitive as other systems. While this is true, it is not a simple issue. ZCA is inherently less sensitive than spectral analysis, mainly because it is not possible to extract a weak signal from noise in a separate frequency band. This often limits the sensitivity to which a ZCA system can be set. A heterodyne system is the most sensitive, although it can not simultaneously monitor all frequencies used by bats, or simultaneously detect bats using widely different frequencies. With time expansion, any sensitivity advantage is offset by the fact that it can only monitor for a small proportion of the time. In practical terms, Anabat is less sensitive, but this doesn't necessarily mean it will detect fewer bats (see Corben and Fellers 2001).

Anabat isn't sensitive enough. This clearly is not the case. I routinely use Anabat for bat-survey purposes, and I don't find the sensitivity to be a problem. Greater sensitivity would be advantageous but it is not necessary, any more than it is necessary to use larger mist nets. What matters is not the sensitivity itself, but rather that sampling strategies are appropriate to the equipment being used. As long as a detector is capable of detecting a particular species, it can be used to sample that species, and Anabat is capable of detecting any known echolocating bat. As with other systems, detection ranges for whispering bats may be very small, but Anabat easily detects other species at distances adequate for general survey purposes.

Anabat doesn't have a flat frequency response. Air does not have a flat frequency response, and the frequency response of air varies with the weather. Thus nothing a detector can do will provide a flat frequency response for the overall signal path from bat to observer. By design, Anabat has a response, which makes it less sensitive to low frequencies. This helps make Anabat effective as a general-purpose instrument. Increasing the sensitivity to low frequencies leads to excessive interference from insects, wind, and other audible sounds. Fortunately, low-frequency bat calls are not strongly attenuated by the atmosphere and tend to be inherently loud, so the bats producing them are usually easily detected.

Anabat is inaccurate. Fenton (2000) criticized the accuracy of Anabat, claiming that its use imposed a sacrifice in this regard. He illustrated alleged discrepancies between the 2 systems he evaluated in Fig. 3 (Fenton 2000). That figure shows Anabat displaying frequencies 13 kHz higher than those displayed by the Pettersson system for the same parts of the same calls. In fact, the accuracy with which Anabat measures time intervals, and therefore frequencies, is dependent on the crystal-controlled oscillators in the hardware, and these provide an accuracy that is at least comparable to that of any spectral-analysis system. Accuracy of either system is easily sufficient for species identification, and neither system would gain advantage from greater accuracy. With properly functioning equipment, the differences depicted in Fenton's Fig. 3 could not possibly be real.

Using either spectral analysis or Anabat, it is necessary to ensure that measurements are made from calls of sufficient quality. For example, it would be pointless to make measurements of maximum frequency from the recordings of either system depicted in Fig. 3 of Fenton (2000), since there is no evidence that the highest detectable frequencies were being recorded by either system. To be meaningful, measurements must be made from adequately recorded calls with equipment functioning properly and used appropriately (Corben and Fellers 2001).

Resolution in Anabat is a more complex issue, which depends on signal frequency, division ratio, and the particular display being used. It might be limited by the number of rows of pixels available for display, or it might be limited by the 1-microsecond resolution of the crystal-controlled time base against which all time intervals are measured. However, it is easily adequate for the purpose of species identification. It isn't necessary to know the frequency of a bat call to the nearest 10th of a percent.

A curious point is that published examples of spectrograms of bat calls are generally depicted with a very coarse resolution. Typical examples can be seen in Parsons and Jones (2000), Fig. 2. In this figure, the two species of pipistrelles are depicted in such a way that any difference in frequency between them is effectively unresolvable. From their Table 2, it seems that the flattest portions of these calls differ in typical frequency from 46 to 52 kHz, a difference easily seen using Analook. My own experience suggests that the underlying reason may be simply that spectral analysis doesn't make it easy to see small differences in frequency without making measurements. If so, this could explain why people using spectral analysis for species identification tend to emphasize the importance of quantitative measurements, whereas Anabat users are more likely to use a qualitative approach.

Anabat measures calls differently from spectral analysis. Start Frequency (Fs), End Frequency (Fe), and duration are all measured differently by Anabat compared to spectral analysis. This results primarily from the use of frequency division, which requires detection of a certain number of zero crossings before any point can be plotted. Likewise, at the end of a call, a number of zero crossings might be detected without being plotted. These differences are not matters of inaccuracy, since the two systems are measuring different things.

To some extent, values measured by Anabat can be adjusted to make them more equivalent to those that would be measured by a spectral-analysis system. These adjustments assume that there was no sudden change in slope at the start or end of the call, but in practice, this requirement can be violated without making much difference. Frequencies and times associated with the first and last pairs of points plotted in a particular call are required, and can be obtained by using the "extract" feature in the file menu of Analook. Given:

F1, F2 = frequencies of 1st and 2nd points, respectively
T2-T1 = time difference between 1st and 2nd points
Fm, Fn = frequencies of second last and last points, respectively
Tn-Tm = time difference between second last and last points

we can estimate:

Fs = 3*F1 - 2*F2
Ts = 3 * (T2-T1)
Fe = 2.5*Fn - 1.5*Fm
Te = 0.5 * (Tn-Tm)

where Fs and Fe are the estimated Start and End frequencies respectively and Ts and Te are the estimated adjustments to be added to the start and end times, respectively. Thus, Ts and Te should both be added to the duration as measured by Anabat to get an estimate of the duration measured by spectral analysis.

There is no point in making these adjustments unless the calls were detected to the maximum extent possible. This situation is indicated if several consecutive calls show similar values for the start and end frequencies, since this indicates that the proportion of the call being detected was unaffected by the changing distance between bat and detector. The same limitation should also be applied to making measurements of start and end frequencies using spectral analysis.

The above differences in measurement have little consequence in my opinion, because they are not large differences compared to the variation bats show in these parameters. However, they should be accounted for when interpreting how calls displayed by one system will look when displayed by the other. Anabat and spectral analysis closely correspond in their depictions of frequency and slope in those parts of a call with the most amplitude. However, such parameters have rarely been employed except by Anabat users. For example, characteristic frequency corresponds to the frequency at the right-hand end of the portion of a call with the lowest absolute slope. It is easily the most useful single feature available for species identification and is relatively unaffected by how far the bat is from the detector. It can also be measured effectively by both systems (Fig. 2).

Anabat "throws away" most of the information in a call. Parsons et al. (2000) assert that "information contained in those parts of the waveform that are not divided is lost, and so is a large part of the frequency-time structure of the signal." This statement misrepresents how frequency division operates, and seems to be based on the misconception that frequency-division systems allow "only every *n*th cycle to pass through" (Parsons et al. 2000). In fact, frequency division produces an output that represents the average frequency over each time interval, so all the frequency-time information is used, not discarded.

The ZCA output is a series of points, which represents how the frequency of the dominant harmonic changes in time, and the question is how well these points depict the frequency-time course of the original signal for the intended purpose, which is to identify bats. In practice, using a frequency-division ratio of 16, most bat calls are well represented and the display of the frequency-time course is adequate. For short-duration, steep calls, too few points might be displayed at a division ratio of 16, though this is rarely a problem for

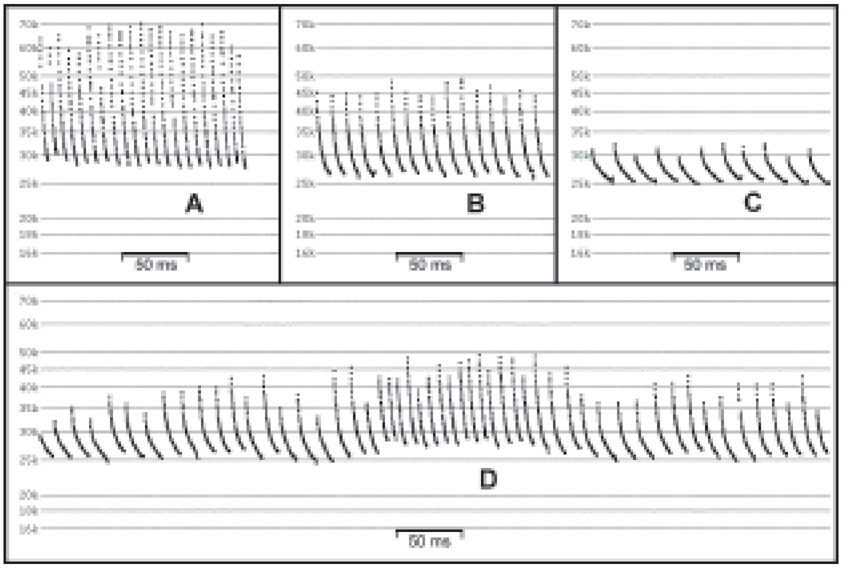

Figure 4: Search-phase sequences of *Eptesicus fuscus*. **A:** Flying in high clutter inside a grove of eucalyptus at Vandenburg Air Force Base, southern California. **B:** Flying in moderate clutter beside trees in the Coconino National Forest in Arizona. **C:** Flying in low clutter over a river at Los Padres National Forest in southern California. **D:** In Sonoma County, central California, flying along an open roadway and passing closer to trees near the middle of the sequence. Note that the type of call is continuously adjusted as clutter changes, and that even at high clutter, extended sequences of similar calls may be produced.

species identification. For those few species which produce such calls at low frequencies (e.g., *Euderma maculatum*), a division ratio of 8 is a better choice (Fig. 3).

It would be possible to show more points in an Anabat display by using a lower division ratio. However, unless these extra points enhanced the probability of correctly identifying a bat, they would not constitute useful information and would simply waste storage space. In general, the features of bat calls do not change suddenly, and so the frequency-time course is adequately depicted by use of an appropriate division ratio. Extremely brief calls, such as those in some feeding buzzes, may not be depicted well by ZCA, but their presence is usually discernible. So far, there is no evidence that depicting feeding buzzes with more detail would aid species identification.

Anabat does not provide a high-quality audible signal. The audible output from an Anabat does not present some of the subtleties contained in time-expanded or heterodyne output. These subtleties are used by experienced observers to help identify bats (Ahlén and Baagøe 1999). However, they are only used by observers in the absence of a real-time frequency display such as Anabat provides. Ahlén and Baagøe assert that "certain qualities are lost" when bat calls are analyzed on a computer to make visual frequency-time displays, but I argue that this remains to be tested. An alternative possibility is that information useful for identification is entirely explained by the frequency-time course of the signal and may be adequately represented by a real-time Anabat display.

Anabat isn't portable enough. This is a valid criticism. Currently Anabat requires a laptop computer for effective active monitoring, though this is no longer necessary for passive recording with the introduction of the Compact Flash storage ZCAIM.

USING ACOUSTICS TO IDENTIFY BATS

The following general observations on using echolocation calls to identify bats in the field come from my extensive use of Anabat, mostly in Australia and North America.

Acoustic identification is often difficult. Acoustic identification of bats is rarely straightforward. Some species are easily and unambiguously identified under any circumstances, but most give calls that can be confused with those of others, even with species that are not close taxonomic relatives. It is important to be aware of how identification issues are affected by the species assemblage in a given area. For example, identifying western pipistrelles (*Pipistrellus hesperus*) in California is usually easy; identifying the same species in certain parts of Texas would be more difficult, because of the co-occurrence of eastern pipistrelles (*P. subflavus*).

Acoustic detection is not always the best approach. Some bats are better surveyed using methods other than acoustics. Townsend's big-eared bat (*Corynorhinus townsendii*), while readily identified acoustically over most of its range, is typically easier to locate for survey purposes by searching potential roost sites. Bats of the genus *Nyctophilus* are typically very difficult to identify acoustically because their calls differ so little between species, but they are readily captured in mist nets or harp traps. Many bats of the family Phyllostomidae are both hard to detect and to identify acoustically, but are easily captured.

The search-phase continuum. Usually, acoustic identification of bats is based on search-phase calls, which can be thought of as echolocation calls produced when the bat is not involved in any other type of behavior, such as pursuit of prey. This makes sense, since search-phase calls are those most often encountered in the field, and they typically show greater differentiation between species than other types of echolocation call. However, search-phase calls of most species vary greatly depending on how far the bat is flying from other objects in its vicinity, a parameter typically referred to as clutter. Thus, a bat flying in a straight line, too far from any other objects to detect echoes from them, can be said to be in zero clutter and will typically produce its flattest, lowest-frequency calls under such conditions. At the other extreme, most bats produce much steeper calls of shorter duration and higher frequency when flying in extreme clutter, that is, in very close proximity to other objects. Fig. 4 illustrates this point with example sequences from *Eptesicus fuscus*.

For practical purposes, search-phase calls form a continuum between the types of calls produced in zero clutter and those produced in extreme clutter, though the

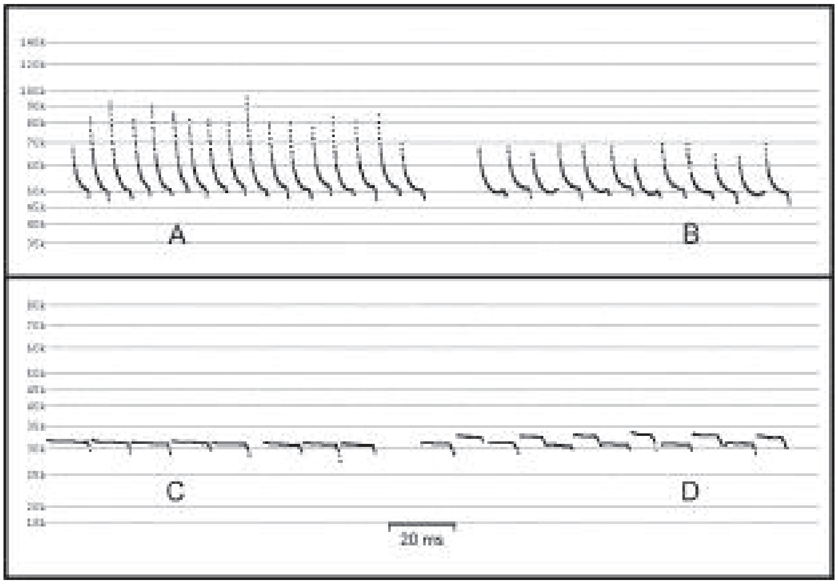

Figure 5: Different modes in search-phase calls. **A:** A typical sequence from *Chalinolobus morio* in moderate clutter. **B:** Another sequence from the same *C. morio* a few seconds later, showing strong alternation between call types. **C:** A low-clutter sequence from *Mormopterus norfolkensis* showing consecutive calls of the same frequency. **D:** Another sequence from the same *M. norfolkensis* a few seconds later, showing strong alternation of call frequencies. Time scale is the same for all sequences.

situation is really more complicated, because many species can switch between different modes of calling (Fig. 5). An appreciation of how search-phase calls vary is critical to the use of these calls in identifying bats.

Diagnostic parts of a repertoire. Although most species produce calls that can be confused with other species, my impression is that a portion of most species' repertoires is diagnostic. These calls are often, but not always, search-phase calls given when a bat is flying in low or zero clutter.

There are many species that typically fly in high clutter, so detection of most diagnostic calls may not be frequent. For example, Yuma myotis (*Myotis yumanensis*) typically hunts low over the surface of water, and therefore in high clutter, while California myotis (*M. californicus*) typically hunts in close proximity to vegetation, again in high clutter. Most of the calls of these two species overlap in frequency and shape with calls of the other species, yet calls given in low clutter differ diagnostically in shape. Thus acoustic identification is facilitated by detection of low-clutter calls, and it is necessary to accept that a very large proportion of calls may go unidentified. Thoughtful placement of detectors could increase the proportion of distinctive calls, if that was an important goal.

In some cases, calls produced in clutter can be more important for identification. An example of this would be the hoary bat (*Lasiurus cinereus*) and pocketed free-tail (*Nyctinomops femorosaccus*), both of which can produce very flat calls at about 17 kHz when flying in zero clutter. However, in more clutter, their calls are quite different, with *N. femorosaccus* producing sequences typical of many Molossids, while *L. cinereus* produces sequences typical of its genus, in which consecutive calls of similar shape jump about in frequency in a distinctive manner.

Avoiding bias in bat-call measurements. A common trend is for calls given in higher clutter to have higher frequencies, higher slopes, and lower durations than those in lower clutter (Fig. 4). One consequence is that call parameters measured from hunting bats will depend on the degree of clutter in which the bat is flying. Thus any factor which influences where a bat tends to fly in relation to its surroundings will also influence the measurements of any calls recorded. This complicates attempts to quantitatively compare the calls of different species or populations, because a bias can be imposed by any correlation between the clutter in which a bat hunts and the categories being investigated.

For example, *Eptesicus fuscus* can be seen hunting in low clutter above the treetops or in very high clutter among tree trunks and close to the ground or at sites of intermediate clutter (Fig. 4). Where the species hunts at any given locality or on any given date could be influenced by many factors, but some such factors might be correlated with geography. This could happen if the hunting behavior of *E. fuscus* is influenced by the presence of other bat species, or if suitable prey items tend to fly at different heights above the ground in different habitats, which in turn exist in different geographical regions. O'Farrell et al. (2000) discussed a case where purported geographic variation may have been at least partly illusory because of a failure to take these considerations into account.

It is important to note that such correlations do not have to be obvious to create problems, and that averages of measured parameters will be affected even by variation in the proportion of time spent hunting in different clutter levels. The resulting biases can be extremely difficult to avoid and may not be apparent to an observer. Furthermore, they will produce statistically significant differences, even though there is no biological difference. The only way to avoid this problem is to understand each species' biology and how it affects the calls they produce, and to then use this information to ensure that appropriate comparisons are made.

Biases from the identification process. Most processes used to identify bats also interfere with them in some way, or otherwise impose biases on the types of calls that may be recorded. For example, despite its usefulness in associating call types with species, recording hand-released bats typically does not adequately represent the range of calls given by free-flying individuals. As a result, call parameters measured from released bats can be expected to differ statistically from those of free-flying animals.

Even the recording of free-flying bats can be subject to such biases, because identification by non-acoustic methods will only be possible under a restricted set of circumstances which limits the range of call types likely to be recorded. For example, with sufficient experience, it is

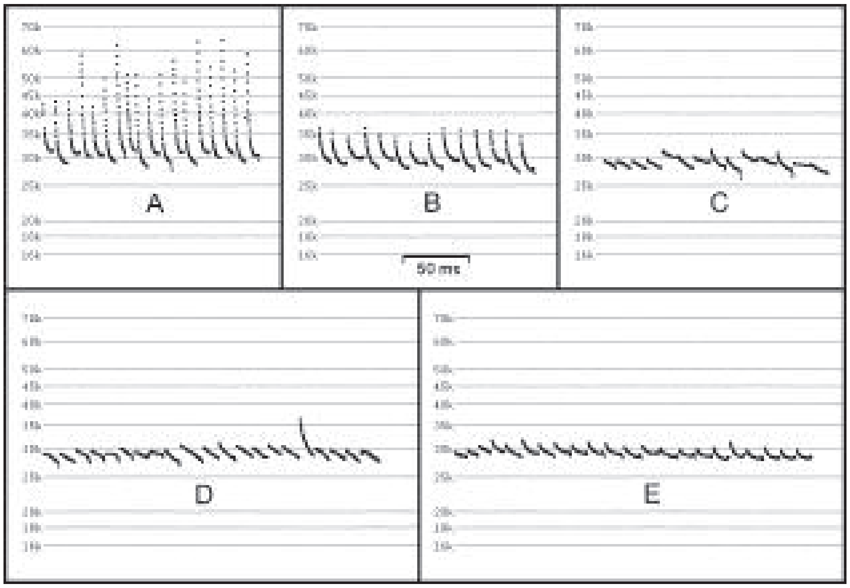

Figure 6: Risk of bias from the identification process. **A** and **B:** show typical sequences of *Chalinolobus gouldii* hunting over roads in moderate and low clutter respectively. **D** and **E:** show different types of sequences of *Mormopterus* spp. hunting above treetops. **C:** Very low-clutter calls of *C. gouldii* flying high over open parking lot. Note first 4 pulses lack alternating frequencies typical of the species, showing that *C. gouldii* is capable of producing sequences closely resembling sequences of *Mormopterus* sp. However, such sequences in *C. gouldii* are rarely encountered and hard to attribute to the species.

relatively easy to visually identify a Gould's wattled bat (*Chalinolobus gouldii*) in typical hunting flight a few meters above the ground using a combination of color pattern, overall shape and size. In such situations, *C. gouldii* will be flying in some clutter and typically produces distinctive sequences in which its calls alternate between higher and lower frequencies (Figs. 6a and 6b). However, *C. gouldii* can produce much flatter calls without frequency alternation when it is flying high in the open (Fig. 6c). Such sequences may be detectable from the ground, but are difficult to associate with the species because the bat is not easy to identify when it is flying so high. If a reference set of calls for *C. gouldii* failed to include such examples, then these calls would most likely be identified as belonging to *Mormopterus spp.* (Figs. 6b and 6c). Once again, adequate understanding of the biology of the animals is essential to avoiding pitfalls of this type.

One consequence of this bias is that criteria developed using calls of known species may not work well when applied to free-flying bats. For this reason, it is important to be wary of statements such as this from Russo and Jones (2002): "The per cent identification rate obtained for each species in DFA ... offers an objective method of determining the probability that a species has been identified correctly." Unfortunately, this ability is well beyond the capabilities of DFA or any other statistical method! The calculated probabilities can only apply to the likelihood that a call belongs to one of the classes to which the DFA has been "trained," They could only apply to the broader categories of species if the reference calls used to set up the DFA adequately represented the variation in all of the species which could possibly be encountered. Since most of the reference calls used by Russo and Jones were recorded from bats leaving roosts, released after capture, or in free flight but under a restricted set of circumstances, it is unlikely that the DFA "training" process will have effectively represented the repertoires of wild bats.

Again, an understanding of the biology of the animals and its effect on their calling behavior is essential before any automated identification procedure will be effective. The "magic bullets" of DFA or neural networks will not solve these problems.

Use of visual observations to obtain reference calls. Even given the limitations outlined above, the recording of bats under real field conditions, when feasible, should provide better reference calls than other approaches. In many cases, a skilled operator can identify flying bats using a combination of visual and acoustic cues, as Ahlén and Baagøe (1999) have described. Features of morphology, color, or behavior often allow for positive identification, though this technique usually requires much practice and self-criticism.

A simple example of how visual observations could be used concerns the Australian yellow-bellied sheathtail bat (*Saccolaimus flaviventris*) and *Mormopterus beccarii*. Acoustic identification criteria for distinguishing these species are not yet well understood (Rheinhold et al. 2001) but they are easily distinguished by sight, because *S. flaviventris* is a much larger bat, with slower wingbeats and a very distinctive, easily observed color pattern. If these bats are identified visually and recorded in free flight, the resulting call recordings will have more relevance to the development of robust identification criteria than any number of hand releases. It is important to remember that the ultimate goal is to identify wild bats, not bats just released from captivity.

Acoustic identification is still in its infancy. Bat acoustic identification is still in an early stage of development, at least in North America and Australia. We have much to learn about the variation within each species and how this variation affects subtle identification issues. Even so, acoustic identification is already a powerful technique, and bat detectors are a vital component for bat surveys. For some species, acoustic techniques are already the most effective tools and should be mandatory whenever the search for those species is required for impact assessment. Which species are best surveyed acoustically is a complex question which must be evaluated on a regional basis, taking into account the potential presence of other species producing similar calls, the ease with which acoustics can be used in relation to other survey methods, and the specific needs of the survey. Such evaluation requires extensive experience with bats and acoustic-survey methods.

Experience is required. The hope is that acoustic monitoring will make surveys for bats simple, even for people with little skill or experience. Unfortunately, this is not currently the case. Even at the simplest level, e.g., in deciding where to place a bat detector, experienced observers perform better, just as experienced practitioners have better success using mist nets.

Bat surveys for environmental-impact assessments often consist of small samples collected by people with little experience. There is little chance that these surveys will detect rare species because it takes skilled operators to know how to effectively locate and identify bats. Wildlife authorities need to understand the importance of imposing protocols that give impact-assessment surveys some chance of meeting relevant goals, and which clearly define where acoustic survey techniques are appropriate and how they should be used.

FUTURE DIRECTIONS

Long-term monitoring is an area with immense potential and the technology already exists to make it work. With access to electrical power, a long-term monitoring station can easily be established using existing Anabat hardware and software and a personal computer, even an obsolete 386. This setup can be used to monitor activity all night, every night. Preliminary data from Gary Fellers' long-term monitoring station at Point Reyes National Seashore, California (Corben and Fellers 2001), underscores how bat activity varies from night to night. It also illustrates how long-term monitoring reveals patterns, such as bat migration, which would be hard to resolve from less comprehensive sampling. A network of long-term monitoring stations has the potential, with sufficient replication, to reveal how bats use a region, how this changes over time, and how it is affected by landscape level changes. Data from long-term monitoring stations, collected using frequency-divided signals, are easily stored and can be reviewed in the future as identification criteria become better established. They could help to provide a baseline for future comprehension of long-term changes.

The establishment of long-term monitoring networks will provide an increased incentive to improve methods for automated identification of species. It is already feasible to use multiple passive-recording stations to record more data than can be economically scanned manually, so automated identification will become a necessity. At present, there are several different approaches to automated identification being investigated, such as the use of neural networks and discriminant function analysis (Parsons and Jones 2000; Russo and Jones 2002) and of synergetic pattern recognition (Obrist et al. 2003). Automated identification has already been successfully applied to field recordings of bats in Victoria, Australia, using Anabat recordings and software written specifically for the purpose by Matt Gibson (L. F. Lumsden, pers. comm.), and an early version of software I have written was used to analyze Gary Fellers' Point Reyes dataset automatically (Corben and Fellers 2001). Even with the current limitations of our understanding of identification criteria, automated identification has value in identifying those species with sufficiently distinctive calls, identifying groups of species with similar calls, and in objectively measuring bat activity from large datasets.

My approach to automated identification involves clarifying what part of a species' repertoire is diagnostic, and concentrating on recognition of calls belonging to that subset. This approach accepts that a large proportion of the recorded calls will not belong to one of these distinctive subsets, and will thus be ignored. Using only a small fraction of the recorded data is not problematic for a ZCA-based system, since accumulation of large datasets is easy. Even the ability to identify just 1% of all calls recorded could still result in enough data to assess patterns of activity for identifiable species. The emphasis is fundamentally different from spectral analysis. Instead of looking at a small number of calls in great detail, ZCA offers the chance to accumulate large datasets through sampling over extended periods at multiple sites, and then to skim those datasets for sequences meeting the needs of automated identification at the resolution required.

In conclusion, zero-crossings analysis is effective for bat-species identification in many circumstances, and in combination with frequency division, is well suited to passive recording. For active monitoring, it offers real-time frequency displays and fast review of previous recordings, using relatively low-power laptop computers. With the advent of more portable equipment, ZCA may surpass the effectiveness of purely auditory methods for surveying bats, because it offers call-sequence storage in combination with an objective assessment of call characteristics from a single event. ZCA's efficiency assures it a continuing role in bat acoustic monitoring, but as with any acoustic-recognition system, it must be used in the context of an adequate understanding of the target species' biology.

ACKNOWLEDGEMENTS

The ideas in this paper came from many discussions with people too numerous to list here, but I am especially indebted to Dixie Pierson and Bill Rainey for their immense help, encouragement, and shared wisdom. I also thank Kim Livengood, Mark Brigham, and Elisabeth Kalko for the work they put into improving earlier drafts.

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Editor's note: Two independent reviewers of this paper remain concerned about the lack of citation to published data supporting many of the author's opinions outlined in the chapter. In the author's defense, logistics prevented him from accessing the literature. While I believe it was essential that this manuscript appear in the volume, I also feel strongly that the concerns of the reviewers should be noted.

BATS IN THE FIELD AND IN A FLIGHT CAGE: RECORDING AND ANALYSIS OF THEIR ECHOLOCATION CALLS AND BEHAVIOR

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Echolocation signals primarily serve to deliver the echo information that a bat needs to solve the different tasks it encounters while commuting and foraging. Many features of echolocation calls consequently are not species specific, but rather situation specific. In a flight cage, it is feasible to standardize some situation-specific variables and therefore to compare the echolocation behavior of different bat species. Behavioral experiments in a flight cage are promising tools to investigate the sensory ecology of bats. They enable us to pinpoint species-specific call parameters for the flight-cage situation, which mimics a cluttered environment. Whether these differences hold for free-flying bats in the field strongly depends on the group of bats being studied. As I show with the genus *Rhinolophus* as an example, frequency parameters measured in the flight tent can easily be used for field identification in bats with "acoustic fovea" and constant-frequency (CF) calls. Many other bats lack this anatomical and physiological specialization and broadcast signals with situation-specific frequency content and duration. In these species, it is impossible to record the whole repertoire of echolocation signals in a flight tent. The genus *Myotis* and other bats that produce frequency-modulated (FM) calls serve to exemplify that signal parameters measured in a cluttered situation can only with extreme caution be used for species identification of bats flying in open space. There, the technically favorable recording situation of a flight cage is compromised by the biological fact of situation-specific variability of echolocation call parameters.

Key words: call repertoire, clutter, echolocation, flight cage, flight tent, *Myotis*, prey capture, *Rhinolophus*, species identification

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INTRODUCTION

Much of our knowledge about bat ecology, behavior, and echolocation comes from studies conducted in the field. Owing to continuous technological improvements in equipment and an ever-increasing number of researchers, bat field ecology is a growing and yet still challenging area of research. In point, this symposium provides an overview of the current state in field-based bat echolocation studies. Another well of information for bat behavioral biologists are psychophysical experiments designed to investigate the performance and sensory capacities of echolocation systems. Often, a 2-alternative forced-choice paradigm is used in which a bat sits on a Y-shaped platform and crawls either onto the left or the right branch of the platform to indicate whether it perceives an object left or right. Such objects might be real targets like thin wires or virtual, so-called phantom targets (i.e., echoes modified by a computer and played back through a loudspeaker).

Whereas field studies allow us to learn about the natural and complex behaviors of bats, they are limited by the difficulty of creating standardized experimental conditions that are necessary to answer many biological questions. In psychophysical tests, the experimental conditions can be controlled, and often reach a high level of sophistication. However, experiments with sitting rather than flying bats and highly specific, sometimes quite simplified psychophysical tasks (for the sake of experimental clarity) are different from a bat's natural foraging situation, where it flies in 3-dimensional space and has to cope with complex echo scenes. In this paper, I discuss flight-cage studies as a method to bridge the gap between field studies and psychophysical experiments. I outline the advantages of working with bats in a flight cage and then address more specifically the advantages and disadvantages of recording echolocation calls in a flight cage. Finally, I address the question of whether acoustic species identifications can be based on flight-cage recordings as a reference database.

WHY WORK WITH BATS IN A FLIGHT CAGE?

If one is interested in the study of hunting and associated echolocation behavior in bats in the field, one has to first know the natural foraging areas of the bats. This is feasible for bats who prefer a specific and delimited habitat type, such as the surface of calm water bodies. Not surprisingly, several detailed field studies report the prey-capture techniques and echolocation behavior of trawling *Myotis* species; those that capture insects from and at low heights above water surfaces (e.g., Britton et al.1997; Jones and Rayner 1988, 1991; Kalko and Schnitzler 1989). Likewise, bats that forage in open situations using echolocation calls with high sound-pressure level can, with considerable effort, be found, observed, and recorded in the field (e.g., Jensen and Miller 1999; Kalko 1995; Kalko and Schnitzler 1993). However, the natural hunting behavior of bats that habitually forage within or close to vegetation and who often use low sound-pressure level echolocation signals is difficult to see in the field. Even radio-tagged individuals are not easy to be directly observed (e.g., Arlettaz 1999; Siemers et al. 1999). It is even more difficult to get close enough to obtain high-quality call recordings especially when the bats are actually capturing prey. For these species, flight-cage studies may provide a suitable approach to observe foraging behavior difficult to observe in the wild (e.g., *Trachops cirrhosus* (Phylostomidae): Barclay et al. 1981; *Plecotus auritus* (Vespertilionidae): Anderson and Racey 1991; *Myotis sp.*: Arlettaz et al. 2001; Faure and Barclay 1992, 1994; Siemers and Schnitzler 2000; Swift and Racey 2002).

Most flight-cage studies focus on gleaning bats and on those which hunt in or close to vegetation. First, only anecdotal foraging observations are often obtainable for these species. Second, flight-cage studies closely emulate natural conditions for these kinds of bats. A flight cage is a restricted and echo-cluttered "habitat" from a bat's point of view. To try and study prey capture behavior of open space foragers in such a restricted environment will not yield meaningful results. However, bats adapted to cluttered habitats should cope well with a flight-cage environment. They can be observed and experimentally tested while performing natural behaviors in this semi-natural setting. If necessary, flight cages can be equipped with habitat elements such as leaf litter, grasses, branches, small ponds, etc. (e.g., Arlettaz et al. 2001; Britton and Jones 1999; Siemers and Schnitzler 2000; Siemers et al. 2001a, 2001b; Swift and Racey 2002).

In a flight cage, it is also feasible to study behavior with a degree of detail difficult to achieve for animals in the wild. This, for example, applies to the study of the bats' motor behavior during prey capture. Close-up photographic or video documentation can be used, as prey can be offered at a predefined location. Detailed behavioral documentation with sufficient sample sizes are relatively easy to obtain. For instance, the role of the tail membrane and feet during prey retrieval from the water surface by *Myotis daubentonii* was clarified in a flight cage (Britton and Jones 1999; Siemers et al. 2001a) complementing previous field observations (Jones and Rayner 1988; Kalko and Schnitzler 1989).

I referred to the difficulty of obtaining quantitative experimental data in the field although there are a range of excellent examples of experimental field studies assessing the sensory basis of food detection with wild bats (e.g., Barclay and Brigham 1994; Boonman et al. 1998; Fuzessery et al. 1993; von Helversen and von Helversen 1999). Two of these studies involved training wild trawling bats to forage in a defined experimental area, selecting and taking objects from the water surface. Von Helversen and von Helversen (1999) worked with flower-visiting glossophagine bats and experimentally manipulated their natural food resource, the inflorescences of the Neotropical liana, *Mucuna holtonii.* Where such field experiments are not feasible, flight cage studies can be used to collect the quantitative data needed. A standardized set up and experimental design allows performance to be compared between species to assess differences in foraging and sensory ecology (e.g., Arlettaz et al. 2001; Kalko and Condon 1998; Siemers et al. 2001b; Thies et al. 1998). If the performance of bats is assessed as a function of the echo-acoustic properties of their environment, natural habitat elements can be acoustically mimicked. For instance, Siemers et al. (2001b) used a linoleum screen to mimic a smooth water surface and a structured polypropylene carpet to mimic vegetation clutter for three species of trawling *Myotis*. The bats searched for prey presented on these backgrounds and, owing to differences in the echo reflecting properties of the background, found far more mealworms on the smooth surface than on the structured one. As an advantage over real vegetation elements, the mimics do not wither or otherwise change over time and therefore provide a standardized experimental setting to different bats and experiments over a long time frame. Ensonification and echo recordings can be used to measure the echo-acoustic properties of the set ups.

Tents can be used as a specific kind of flight cage. There are several brands available. They were originally designed for garden parties; and they make comfortable portable bat labs for the field. They usually have a waterproof nylon roof, ideal to protect bats, equipment, and researchers from rain. The gauze walls are designed to keep mosquitoes out, but they also keep bats and live insects in. Because the tents have no floor, they can be built in and over a wide range of habitat patches and may therefore include a piece of meadow or shrub. Lightweight tents can be carried easily and erected at the location where the bats are captured (e.g., Kalko and Condon 1998; Siemers et al. 2001b). Therefore, bats can be tested right after capture and released afterwards at the site of capture without the additional stress of transportation. Furthermore, it is often easier to gather natural prey for experiments on-site than to collect it further away.

In contrast to most psychophysical experiments, flight-tent studies are mostly designed for newly captured, experimentally naïve bats. No long training is necessary (or wanted), and the bats can be released to the wild at the conclusion of the experiment. If tasks resemble the bats' natural situation, behavior close to natural can be recorded. I recommend minimizing the time bats are kept in captivity (one to several nights) both for the bats' sake and to minimize learning. However, it is hard to completely exclude conditioning to experiment-specific cues even during short experiments. This applies especially for gleaning bats, since quick though transient associative learning of prey-specific situations might be part of their natural foraging behavior (Siemers 2001). Additional flight-tent experiments are needed to test this hypothesis.

The studies I cite here and many others indicate that behavioral experiments in a flight cage are a promising tool to investigate the sensory ecology and prey-capture behavior of many species of bats. A second question is whether they are also suited to record and analyze echolocation calls?

ECHOLOCATION CALL RECORDINGS IN A FLIGHT CAGE AS COMPARED TO FIELD RECORDINGS

There are several advantages to recording echolocation in a flight cage compared to natural situations. The first and most trivial I have already mentioned: Some species are very difficult to observe in the field, especially during foraging and prey capture. To record echolocation behavior in gleaning bats generally will require flight-cage studies. There are, however, some exceptions, where researchers have found favorable conditions to observe and record gleaning attacks by bats in the wild (e.g., Jones and Rayner 1989; Miller and Treat 1993; Schumm et al. 1991).

Second, recording quality can be optimized in a flight tent. This mainly applies to "highly cluttered space bats" (Schnitzler and Kalko 2001), as they often use low amplitude echolocation calls (Waters and Jones 1995) that are severely affected by spreading losses. Geometric attenuation reduces the amplitude of the whole call (6 dB per doubling of distance), whereas atmospheric attenuation mainly affects the higher frequency components. Therefore, in particular the high starting frequency of FM calls is not picked up in unfavorable recording situations. This is also due to the directionality of both the emitter (the bat) and the receiver (the microphone). Directionality increases with frequency. For good (i.e., on axis-) recordings, the bat is flying directly towards the microphone, so its emission beam matches the directionality characteristics of the microphone as closely as possible. It is easier to create these recording situations in a flight tent than in the field. If prey is presented to a bat, the microphone can be placed directly behind to obtain an optimally recorded sequence of approach calls. If the bat adopts a stereotyped flight path in the flight cage, the microphone can likewise be positioned to get on axis-recordings from a part of this flight path. It is possible to observe the bat visually or by using infrared illumination and video recordings synchronized with the sound recordings of its echolocation calls (e.g., Siemers et al. 2001c). This way the calls can be selected that were broadcast by the bat when it flew directly towards the microphone.

A third advantage of using a flight cage is that it is easier to get recordings from known individuals. When individually known bats are flown one at a time, then there is no doubt which individual has broadcast the calls. In contrast, for bats in the field, it is not always easy to identify the caller. Transponders can be used to mark bats in the field (e.g., Kerth et al. 2000), but this technology is limited to close-range identification. Currently, recordings of clearly identified individuals (age, sex, mass, etc.) are usually restricted to one sequence of "release calls" per individual: captured bats are released

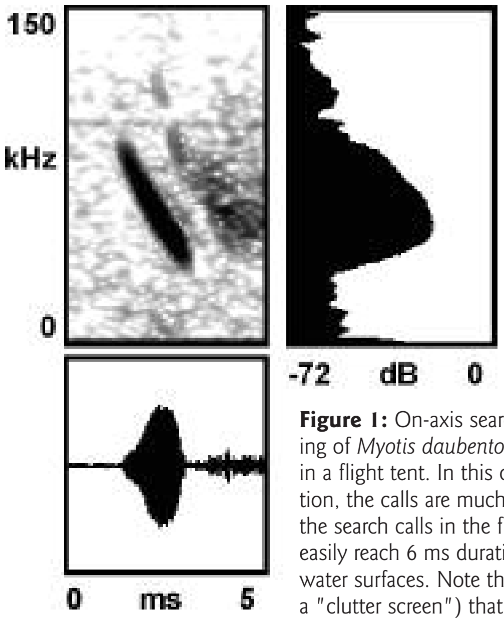

Figure 1: On-axis search call recording of *Myotis daubentonii* (Germany) in a flight tent. In this cluttered situation, the calls are much shorter than the search calls in the field, which can easily reach 6 ms duration over open water surfaces. Note the echoes (from a "clutter screen") that follow the call. Figure adapted from Siemers (2000).

from the hand and fly away towards a microphone. It is necessary for various purposes to record calls of identified individuals: 1) To quantify individual variability in call structure e.g., in sequences of approach calls. 2) To investigate relationships between call structure and life history traits (sex, age, size; Jones et al. 1992, Russo et al. 2001). 3) To search for individual signatures in bat echolocation calls that are potentially useful for intraspecific communication. 4) To avoid pseudo-replication by scoring recordings of a given individual more than once without knowing it.

Fourth, in a flight-cage study, it is possible to evaluate differences in echolocation calls between bat species whose calls are otherwise easily confounded. This can be achieved by taking advantage of the standardized situation bats encounter in a flight cage. It is important to remember that echolocation signals primarily serve to deliver the echo information that a bat needs to solve different sensory tasks encountered while commuting and foraging. Many features of echolocation calls are consequently not species-specific, but rather situation-specific (for review see Fenton 1990; Neuweiler 1989, 1990; Schnitzler and Kalko 2001; Schnitzler et al., in press). It is well known, for example, that bats producing FM calls increase call duration and often decrease starting frequency when the distance to background targets increases (see reviews above and Jensen and Miller 1999; Kalko 1995; Siemers and Schnitzler 2000; Siemers et al. 2001c). In a flight cage, situation-specific variables can be standardized to directly compare echolocation behavior of different bat species. For example, flight-cage recordings yielded consistent differences in search calls of several European *Myotis* species (Siemers 2000; Waters and Jones 1995), which produce rather similar calls and represent a major challenge for acoustic species identification in the field (Parsons and Jones 2000; Vaughan et al. 1997). A considerable amount of variation, however, is attributable to differences between individuals (Siemers et al. 2001b, Siemers, unpublished).

It is important to stress again that all flight-cage recordings are recordings of bats in a cluttered environment. In this situation, bats producing FM calls, typically broadcast short calls (1-4 ms) with large bandwidth (example in Fig. 1). Situation-specific variability is reduced and species- or individual-specific differences between these short, broadband calls can be analyzed. This advantage of flight-cage recordings is at the same time its major disadvantage: In a flight cage, bats do not broadcast the type of echolocation call that they use when flying in open space high aboveground. There is usually a gradual change in call structure from cluttered to uncluttered situations (for review see, e.g., Fenton 1990; Neuweiler 1989; Schnitzler and Kalko 2001; Schnitzler et al., 2003); i.e., bats do not broadcast one standard call, but employ a repertoire of echolocation calls. This brings us to the final question of whether acoustic species identifications can use flight cage recordings as a reference database.

CAN ACOUSTIC SPECIES IDENTIFICATIONS USE FLIGHT CAGE RECORDINGS AS A REFERENCE DATABASE?

In a flight cage, it is impossible to confront a bat with all potential habitat types found in the wild because a flight cage only mimics a cluttered environment. Therefore, only recordings of one end of the call repertoire, the types of calls broadcast in cluttered situations, can be made. The question arises whether these calls contain enough species-specific information to predict the call pattern of the respective species in other situations. The answer depends strongly on the group of bats being studied.

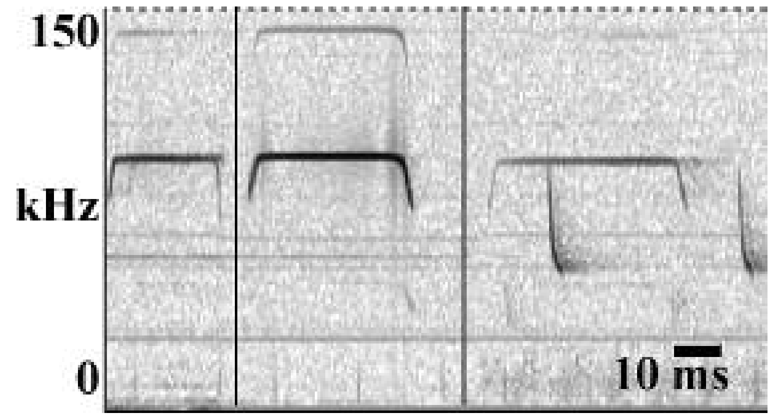

Figure 2: Echolocation calls of *Rhinolophus blasii* (Bulgaria) from three different behavioral situations show similar (but not equal: individual variation + Doppler compensation) CF frequency. From left to right: flight cage, handheld, and in the field. Note the two Miniopterus FM calls on the field recording.

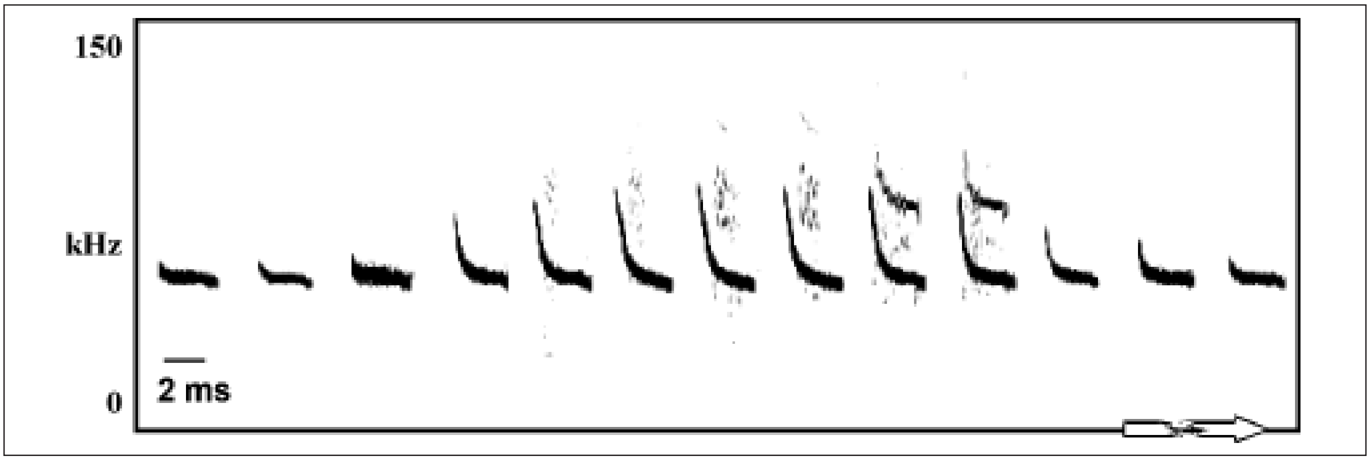

Figure 3: Spectrogram representation of *Myotis nigricans* (Panama) search calls: Individual *M. nigricans* change from narrow band to broadband and back to narrow-band signals again, as the individual flies from open space to a forest edge and back into the open again. Note that the pulse intervals have been cut out. Figure adapted from Siemers et al. 2001c.

Bats of the Old World families Rhinolophidae and Hipposideridae and the Neotropical mormoopid *Pteronotus parnellii* produce long (> 20 ms; hipposiderids > 10 ms) constant frequency (CF) calls with high duty cycle (Fig. 2). They are characterized by anatomical and physiological specializations of the hearing system that allows a fine frequency resolution around their CF frequency – the so-called "acoustic fovea" (for review see Moss and Schnitzler 1995). These specializations are used to detect fluttering prey by evaluating the "acoustic glints" (rhythmical amplitude and frequency shifts) modulated onto the echoes by insect wing beats (Schnitzler 1987). Therefore, these bats always center the returning echoes in the high-resolution frequency range of the acoustic fovea. When hanging from a perch, they emit their CF call component at their "resting frequency". When flying, they compensate for frequency shifts caused by Doppler shift due to their own flight speed by lowering the emission frequency by up to 3 kHz, depending on flight speed and absolute value of resting frequency (reviewed in Moss and Schnitzler 1995). In essence, there is variability in CF frequency within individuals caused by Doppler compensation, and there is individual variation within a population (e.g., Jones et al. 1992; Russo et al. 2001). However, this variability is not situation-specific. Call duration varies with the echolocation task faced, but emitted CF frequency is the same at different distances to clutter (rhinolophids and hipposiderids usually do not fly in open space), in order to match the returning echoes with the acoustic fovea. This means that CF frequency parameters measured in the flight tent can be used for field identification in rhinolophids and hipposiderids. Depending on the distribution of CF frequencies among rhinolophid and hipposiderid species in the local community, this might or might not allow unequivocal identification of wild bats from their CF frequency (e.g., Heller and von Helversen 1989, Kingston et al. 2000; Russo et al. 2001; Siemers, Dietz, Schunger and Ivanova, unpublished data on Bulgarian *Rhinolophus* community). In the case of rhinolophids and hipposiderids, field biologists benefit from the biological specialization of the acoustic fovea and associated CF constancy, such that flight-tent data, can be used for field identification.

Most bats lack the anatomical and physiological specialization of an acoustic fovea and broadcast signals with situation-specific frequency content (and duration). Likewise, these differences have an adaptive value and serve to solve different orientation tasks (Fig. 3; for review see Fenton 1990; Neuweiler 1989, 1990; Schnitzler and Kalko 2001; Schnitzler et al., 2003). For these species, it is impossible to record the entire species repertoire of echolocation signals in a flight tent. In a tent, they typically produce short broadband signals (< 4 ms, > 40 kHz frequency range), whereas in open space they might well broadcast long and narrowband signals (> 10 ms, < 10 kHz frequency range). The natural transition in calls by bats moving from clutter to open space is usually characterized by a gradual change in call parameters. While (in downward FM calls) duration gradually increases and starting frequency gradually decreases, terminal frequency remains relatively constant for many species. In some species terminal frequency decreases slightly with increasing call duration; generally by < 5 kHz across the search-call repertoire. Additionally, terminal frequency is less affected by atmospheric attenuation than the higher starting frequency. Consequently, field recordings give more reliable measurements of terminal frequency, as they are less dependent on the distance and the angle of the bat to the microphone than starting frequency.

For both, biological and technical reasons, terminal frequency recorded in a flight cage is the most suitable parameter to use to predict natural signal structure in open space. This is of no use for species that introduce new call types when flying in the open (e.g., *Barbastella barbastellus*: Denzinger et al. 2001; Parsons and Jones 2000). But even such a prediction based on terminal frequency does not provide a sound basis for field identification, and I reiterate that, for many species, field discrimination depends on a comprehensive reference database that is not collected solely in a flight cage.

SUMMARY

To summarize, flight-cage studies

- are useful for investigating foraging, prey capture and echolocation behavior by bats adapted to cluttered situations.
- yield high-quality call recordings.
- are useful for establishing intra- and interspecific call variability in a standardized situation of a cluttered environment.
- are, for most species of bats, of limited use to determine the entire full call repertoire as a comprehensive reference database for acoustic field identification.

ACKNOWLEDGMENTS

I want to thank Bat Conservation International for inviting me to this inspiring meeting. The constructive suggestions of Mark Brigham and Elisabeth Kalko were most welcome for revising this manuscript. I thank Hans-Ulrich Schnitzler for constant support and the German Science Foundation (DFG Si 816/2-1) for funding part of my research.

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SIGNAL PROCESSING TECHNIQUES FOR SPECIES IDENTIFICATION

AUTOMATED SPECIES IDENTIFICATION OF BATS IN THE UNITED KINGDOM: A CASE STUDY

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In this paper, I describe a case study in which echolocation calls of 14 species of bat known to breed in Britain were characterized using an automated process. The paper describes the signal-processing techniques used to characterize the signals and explains why they were used. After characterization, the calls were classified to species level by discriminant function analysis and artificial neural networks. For almost all species, the artificial neural networks outperformed the discriminant analysis due to their error-reduction mechanisms. The results are discussed in terms of the requirements of automated species identification systems and their application and the different properties of discriminant analysis and artificial neural networks.

Key words: artificial neural networks, automated, multivariate statistics, signal processing, species identification

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INTRODUCTION

The analysis of sound signals requires the use of specific mathematical techniques. The number of techniques available is almost endless; to those not trained as engineers, however, it is difficult to assess which is best. Luckily, most biologists only need to use a small number of these techniques, and several companies produce software to assist researchers with signal analysis. Signal-processing techniques represent an extremely powerful tool when exploring sound signals, but like every powerful tool, they are easy to misuse. In the case of acoustic-identification systems for bats, this misuse often leads to incorrect identification of species. In situations where conservation and/or management decisions are determined based on identifications, the consequences of a mistake can be far-reaching and expensive. It is vital, therefore, that researchers have a basic understanding of signal-processing techniques and how to apply them correctly. This paper will use a case-based approach to show how signal-processing techniques can be used effectively for acoustic species identification of bats. The case in question is a research project that began at the University of Bristol in 1998 to develop new methods for identifying bats based on their echolocation calls.

Since the early 1990s, researchers at the University of Bristol have been evaluating systems for identifying bats from echolocation calls. Prior to 1998, systems were based on time-expanded echolocation calls analyzed using 'traditional' multivariate statistical techniques such as discriminant

Species (common name)	N	Start Frequency (kHz)	End Frequency (kHz)	Frequency with Most Energy (kHz)	Duration (ms)	Inter-Pulse Interval (ms)
Barbastella barbastellus (Barbastelle)	21	40.3 (± 3.4)	30.2 (± 4.1)	34.9 (± 3.5)	3.1 (± 0.7)	63.8 (± -)
Eptesicus serotinus (Serotine)	56	59.5 (± 7.1)	27.6 (± 1.9)	32.2 (± 2.8)	6.9 (± 2.3)	116.0 (± 26.2)
Myotis bechsteinii (Bechstein's bat)	25	109.2 (± 11.9)	33.8 (± 3.9)	51.0 (± 3.1)	2.1 (± 0.4)	96.4 (± 13.1)
M. brandtii (Brandt's bat)	50	85.5 (± 13.3)	33.7 (± 3.8)	47.9 (± 8.3)	3.1 (± 0.8)	88.0 (± 18.3)
M. daubentonii (Daubenton's bat)	24	84.2 (± 4.8)	29.4 (± 3.7)	46.2 (± 4.5)	2.9 (± 1.0)	78.6 (± 22.6)
M. mystacinus (Whiskered bat)	37	80.3 (± 12.1)	32.2 (± 3.4)	47.5 (± 5.9)	2.2 (± 0.9)	87.8 (± 23.8)
M. nattereri (Natterer's bat)	82	121.4 (± 10.2)	22.5 (± 5.1)	51.2 (± 11.3)	2.3 (± 1.1)	76.8 (± 24.8)
Nyctalus leisleri (Leisler's bat)	80	36.3 (± 10.3)	25.5 (± 1.6)	28.9 (± 2.0)	10.7 (± 1.7)	177.0 (± 71.6)
N. noctula (Noctule)	47	25.9 (± 7.2)	18.2 (± 2.1)	20.3 (± 2.0)	19.6 (± 4.4)	336 (± 138.0)
Pipistrellus pipistrellus (Bandit Pipistrelle)	36	71.3 (± 14.0)	43.3 (± 1.8)	46.0 (± 1.8)	4.8 (± 1.3)	98.2 (± 32.1)
P. pygmaeus (Soprano Pipistrelle)	96	83.2 (± 17.0)	52.2 (±1.7)	53.8 (± 1.7)	5.0 (± 1.1)	95.2 (± 27.5)
P. nathusii (Nathusius' Pipistrelle)		49.8 (± 6.8)	36.7 (± 1.0)	39.3 (± 1.0)	6.1 (± 1.2)	134.0 (± 55.9)
Plecotus auritus (Brown Long-Eared bat)	32	49.9 (± 3.1)	27.1 (± 1.8)	53.1 (± 14)	1.8 (± 0.5)	71.3 (± 25.0)
Pl. austriacus (Grey Long-Eared bat)		41.7 (± 2.2)	21.4 (± 1.7)	28.0 (± 1.3)	4.6 (± 1.2)	105.0 (± 21.5)
Rhinolophus ferrumequinum (Greater Horseshoe bat)	24	69.4 (± 1.7)	67.5(± 3.4)	82.1 (± 0.5)	51.6 (± 12.4)	83.0 (± 18.9)
R. hipposideros (Lesser Horseshoe bat)	33	97.5 (± 1.6)	96.1 (± 9.3)	111.5 (± 1.1)	40.8 (± 10.5)	82.4 (± 12.3)

Table 1: Species of bats known to breed in the United Kingdom and average values for parameters measured from their echolocation calls. Sample sizes are given for calls analyzed as a part of this study.

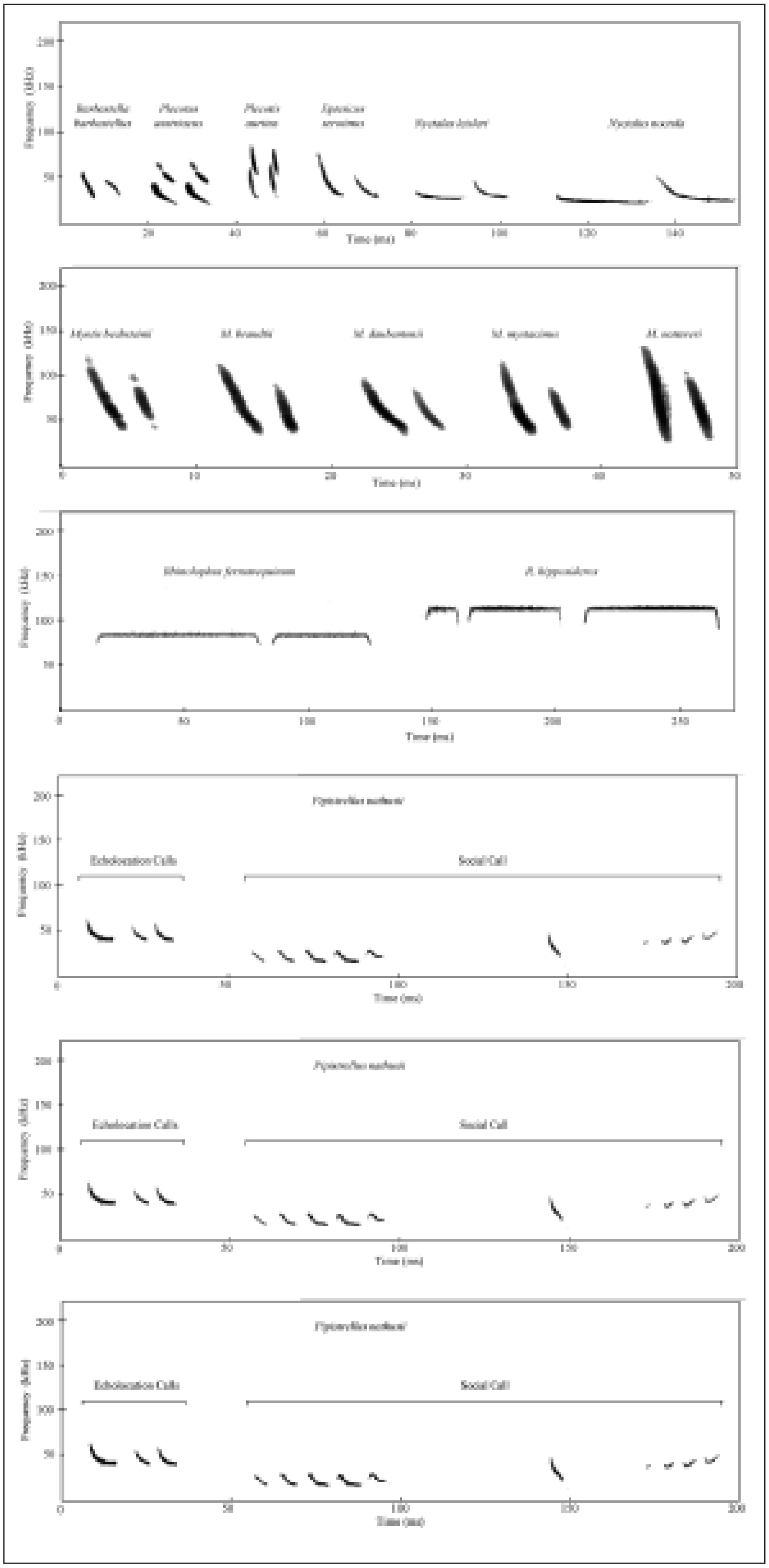

Figure 1: Spectrograms of search-phase echolocation calls and social calls (for *Pipistrellus* spp. only) of United Kingdom bats. Each pair of calls represents the extreme variation shown by that species. Note that each figure uses a different time scaler.

function analysis. In 1998, it was decided that a new approach was needed. Previous systems had been based on the notion that echolocation calls need be analyzed 'by hand.' Although analyses were always carried out with the utmost care, they were subject to a varying degree of user bias. Discriminant function analysis, although a powerful mathematical technique, also has its limitations. The focus of the new approach was threefold: 1) to develop a system that was as free from user bias as possible, mainly through automation of call analysis; 2) to use newer and more powerful classification techniques, such as artificial neural networks; and 3) to move away from recording techniques that could not be carried out in 'real-time.'

It is the aim of this paper to detail the methods used to develop the new species identification system, and the rationale for the techniques used.

BATS (AND ECHOLOCATION CALLS) OF THE UNITED KINGDOM

All 16 species of bat known to breed in the United Kingdom use echolocation for orientation and prey detection. Nine species can be considered difficult to identify acoustically due to having call parameters that overlap with those of at least one other species.

The bat fauna of the United Kingdom is dominated by the 14 species of family Vespertilionidae (Table 1). The remaining two species belong to the family Rhinolophidae. With the exception of the rhinolophids, all United Kingdom bats produce short- to moderate-duration frequency-modulated (FM) search-phase echolocations calls (Fig. 1). Most broadband calls, such as those produced by *Myotis nattereri*, can sweep from 150 kHz to 17 kHz. At the other extreme, the calls produced by *Nyctalus noctula* only sweep through 5 kHz on average. Call durations are also highly variable, ranging from 2 ms (*Plecotus auritus*) to 20 ms (*N. noctula*). The two species of *Rhinolophus* produce long-duration (ca. 50 ms) constant-frequency calls (CF), beginning and ending with FM sweeps. As with all bats, the structure of echolocation calls is context specific and therefore highly flexible.

RECORDING OF ECHOLOCATION CALLS

Before developing a species identification system, a library of echolocation calls from known individuals of each species must first be compiled. Calls should be recorded in as natural a situation as possible. Ideally, bats should be recorded in unhindered flight and in the habitat in which they are most commonly encountered or in the habitat in which they produce characteristic echolocation calls. For our project, calls from most individuals were recorded at flyways leading away from roost sites known to contain only one species. Where roosts could not be identified, or it was not possible to make accurate recordings near a roost, bats were recorded after release from the hand following capture by mist net or harp trap. Releases were always carried out in the same habitat in which the bat was captured. We recorded calls as long after release as possible so that they were as close to those made during normal flight as possible (i.e., after the bat had oriented to its surroundings and was a sufficient distance from the point of release). To avoid recording the same bats over several nights, only calls made on one night were analyzed. Calls were recorded from as far away from the roost entrance as possible while still being able to ensure that the bats had emerged from that roost. Recordings were usually made on-axis as the bat flew towards the microphone. As high-frequency sounds are highly directional, recordings made off-axis may not represent the call in the form it was emitted by the bat (Pye 1993). Equally, calls recorded a long distance from the bat will be highly attenuated, with high frequencies affected to a greater degree (Lawrence and Simmons 1982). This can have the effect of decreasing the bandwidth and duration of calls. We recorded calls from a total of 698 individuals. Of the bats known to breed in Britain, only *Pipistrellus nathusii* and *Plecotus austriacus* were not recorded.

Two different recording methods were used. Initially, we used a bat detector (UltraSound Advice S-25, UltraSound Advice, London, United Kingdom), linked to a time-expansion unit (UltraSound Advice Portable Ultrasonic Processor (PUSP)). The PUSP, when triggered by the user, digitized a 2-second sequence of sound. Sequences were sampled at 448 kHz with 8-bit precision and time-expanded by ten times before being recorded to a Sony Professional Walkman (model WM-D6C). From early 1999, we digitized echolocation calls directly to a laptop computer (i.e., not time expanded). Using the S-25 bat detector, coupled to a National Instruments PCMCIA-format analogue-to-digital (A:D) conversion board (model AI-16E-4, National Instruments, Austin Texas), 5-second sequences of sound were acquired. This method allowed calls to be recorded in real time.

DIGITIZATION AND SIGNAL CONDITIONING

Time-expanded signals recorded using the Walkman were digitized to a computer using the BatSound software package (Pettersson Elektronik AB, Uppsala, Sweden) at a sampling rate of 44.1 kHz (effective rate: 441 kHz) using the standard sound card contained in a Toshiba Satellite Pro laptop computer (model 4080XCDT, Toshiba of Europe, London, United Kingdom). When using the National Instruments conversion board, recording and digitization occurred simultaneously. Signals were digitized when the system was triggered by the user, using BatSound, at a sampling rate of 500 kHz, with 12-bit precision.

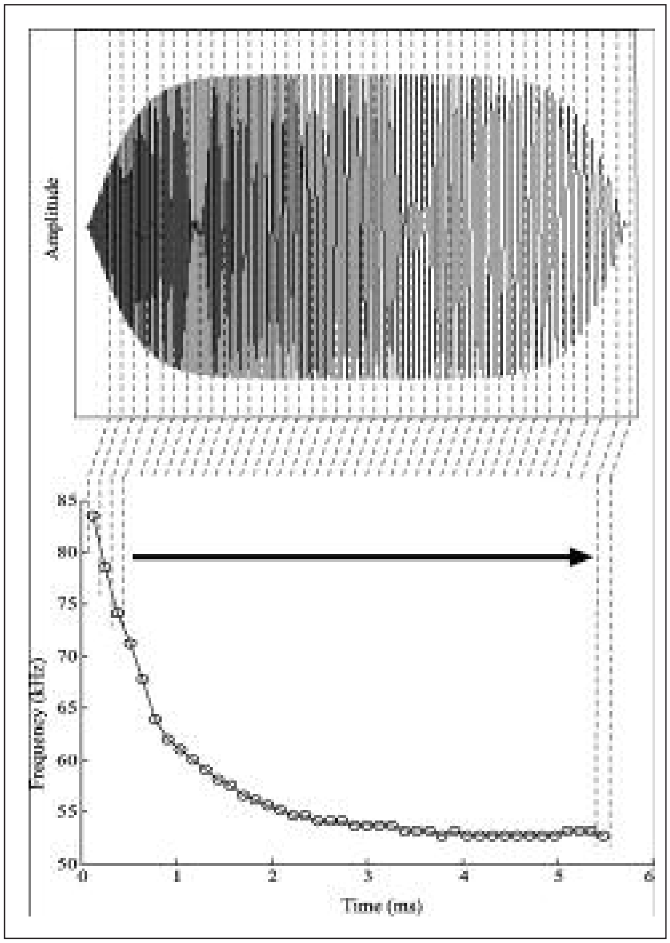

Figure 2: Waveform and the resulting reconstruction of an echolocation call's frequency-time course using the sliding power spectrum method. Blocks of 56 data points from the waveform were zero-padded to 1,024 points and a power spectrum was then performed. Data points on the frequency-time course reconstruction represent the frequency with most energy from each of the power spectra.

Using BatSound, a single call from each sequence with the highest signal-to-noise ratio, without being overloaded, was subjected to further analysis in MatLab v5.3 (Mathworks, Natick, Massachusetts). A mid-pass (10-150 kHz), 10^{th} order Butterworth filter was applied to signals.

AUTOMATED CALL EXTRACTION AND MEASUREMENT OF CALL PARAMETERS

The envelope of each digitized signal was calculated using a Hilbert transform of the waveform. Envelopes were subsequently scaled between 0 and 1. Using the point where the envelope rose above and then subsequently dropped below an arbitrary threshold value (0.0005), the call was removed from the rest of the signal. Call duration was defined as the duration of the extracted waveform. The frequency with most energy was measured from a 1024-point power spectrum (frequency resolution of 430 Hz and 488 Hz for signals digi-

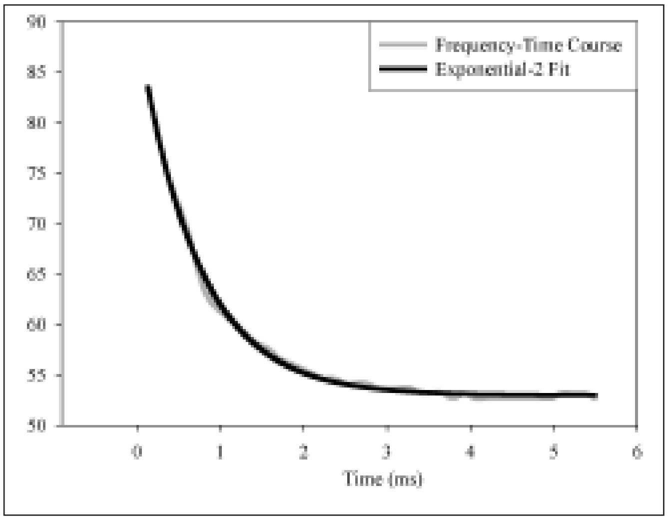

Figure 3: Reconstructed frequency-time course of an echolocation call showing the approximation given by an exponential 2-curve function. The difference between the two curves is measured using the mean-squared error of the difference.

tised at 441 kHz and 500 kHz respectively). A Hamming window was applied to the call before the power spectrum was calculated. The frequency-time course of the harmonic with most energy was recreated by dividing the call into a series of 56-point segments (Fig. 2). We calculated power spectra for each segment (after the application of a Hamming window) using a zero-padded 1024-point fast fourier transformation (FFT), and the frequency with most energy calculated within each power spectrum. This gave a frequency versus time reconstruction of the call at 127-microsecond and 112-microsecond intervals when digitized at 441 kHz or 500 kHz, respectively. Each reconstruction was checked visually to ensure there were no obvious errors. The start and end frequencies of the call were taken to be the first and last values from the reconstructed call. The frequency at half the duration was also taken from the reconstruction.

With the exception of *P. auritus* calls, the harmonic with most energy within each call was always measured. At the beginning of a *Plecotus* call, most energy is usually in the fundamental, after which it can switch to the second harmonic. For the sake of consistency in our measurements, we chose to measure all variables, except frequency with most energy, from the fundamental of *P. auritus* calls. Frequency with most energy was measured across all harmonics for all species.

We fitted eight mathematical curve functions to the reconstructed frequency-time course of calls using a non-linear regression procedure in MatLab's statistics toolbox (v2.2; Fig. 3). The eight functions were: linear, logarithmic, hyperbolic, exponential-1, exponential-2, power-1, power-3, and parabolic. Mathematical descriptions of each of the functions are given in Parsons and Jones (2000). Three of the curves (hyperbolic, logarithmic, exponential-1) are the same as those used by Masters et al. (1991) and Parsons et al. (1997). The mean-squared error of the difference between the curve function and the true frequency-time course of the call was used to measure the goodness of fit of each function.

TRAINING AND TESTING OF ARTIFICIAL NEURAL NETWORKS

To test the ability of artificial neural networks to classify echolocation calls to species level, we trained multilayer perceptrons using a back-propagation algorithm with momentum (e.g., Haykin 1999; Rumelhart et al. 1986), epoch training, and adaptive learning (Vogl et al. 1988) using Matlab v5.3's neural network toolbox (toolbox version 3.01).

We trained a hierarchical system of networks to classify calls. Parameters from a digitized call were first identified to genus level by one network, and then by a second network to species level depending on the result of the genus-level classification (Fig. 4). In all cases, the inputs to the networks were the five temporal and spectral call parameters and eight mean-squared errors from the curve fitting analysis. The network outputs were the species emitting the calls to be classified. The number of hidden layers, the number of hidden neurons within

A

True Group

Classified As	*M. bechsteinii*	*M. brandtii*	*M. daubentonii*	*M. mystacinus*	*M. nattereri*
M. bechsteinii	9	2	0	1	2
M. brandtii	0	21	0	1	1
M. daubentonii	1	1	9	1	0
M. mystacinus	2	1	3	14	1
M. nattereri	0	0	0	1	37
% Correct	75%	84%	75%	78%	90%

B

True Group

Classified As	*N. leisleri*	*N. noctula*
N. leisleri	39	1
N. noctula	1	43
% Correct	98%	98%

C

True Group

Classified As	*P. pipistrellus*	*P. pygmaeus*
P. pipistrellus	18	0
P. pygmaeus	0	48
% Correct	100%	100%

Table 2: Results from three neural networks trained to identify the calls of bats belonging to the genera **A:** *Myotis*, **B:** *Nyctalus*, or **C:** *Pipistrellus*.

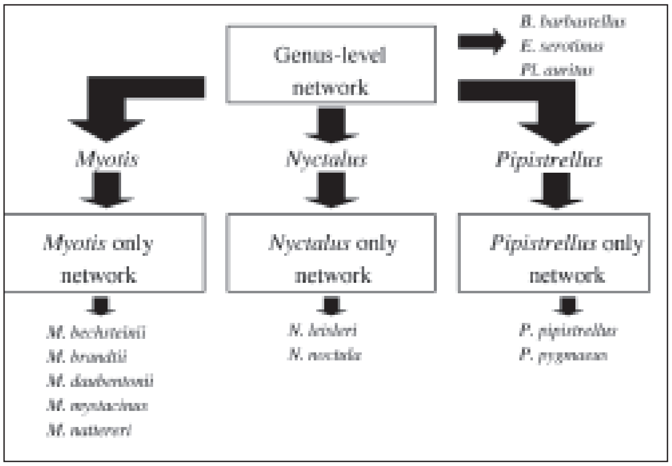

Figure 4: System of hierarchical artificial neural networks used to identify the species of bat producing an echolocation call.

each, and the momentum constant used varied depending on the classification task (e.g., to genus level, *Pipistrellus* spp., etc.). Networks were trained using 50% of the data set. Prior to being split, data were randomized within each species to remove any effect of recording equipment, year of recording, recording site, or geographic variation in call structure. After training, the remaining 50% of the dataset was used to independently test the networks.

To allow comparison of results from the artificial neural networks with techniques developed at the University of Bristol and published in the literature, calls were also classified using discriminant function analysis.

RESULTS OF CLASSIFICATIONS BY ARTIFICIAL NEURAL NETWORKS

The network trained to classify calls to genus level achieved an overall correct identification rate of 96% (Fig. 5). Species within the genera *Myotis* and *Pipistrellus* were identified without error. Only calls from *Eptesicus* sp. had an identification rate below 90%.

The first species-specific network, trained to classify calls produced only by *Myotis* spp., achieved an overall successful classification rate of 82% (Table 2a). The calls of *M. nattereri* were classified with a success rate of 90%, being confused with those of all other *Myotis* spp. except *M. daubentonii*. Eighty-four percent of calls recorded from *M. brandtii* were correctly classified. The calls of *M. brandtii* were confused with all of the other *Myotis* spp. except *M. nattereri*. The correct identification rate of calls recorded from *M. mystacinus* was 78%, while 75% of calls recorded from *M. bechsteinii* and *M. daubentonii* were correctly identified.

The second genus-specific network was trained to identify calls recorded from *N. leisleri* and *N. noctula* (Table 2b). Only one call from each species was misidentified, giving correct identification rates for each – and an overall correct identification rate – of 98%.

The third network, trained to identify the calls from *P. pipistrellus* and *P. pygmaeus*, correctly identified all the calls recorded from both species (Table 2c).

For all networks, correct identification of recorded calls was equal to or higher than that achieved by discriminant function analysis.

DISCUSSION

An obvious question that presents itself when designing or training an acoustic-identification system is: "How many calls do I need to have in my library?" There is not a fixed number of calls that the library must contain before it is considered adequate or complete. In the case of echolocation calls of bats, the library of recordings for any one species must not only contain the characteristic or normal range of calls of that species, but also the extremes that are likely to be encountered by a researcher trying to implement the system in the field. Both discriminant function analysis and artificial neural networks perform poorly when presented with data they have not seen before. It should also be noted that the class of network I describe in this paper has no output category for "I don't know." All calls presented to the trained network will be classified. Calls from species not known to the network should be classified with a very low confidence and so be rejected by the researcher as an unreliable result. However, this may not always be the case. Bat species with similar morphologies and ecologies may produce similar echolocation calls (e.g., *M. mystacinus* and *M. brandtii*). If a network has been trained to identify one species and not the other, presentation of calls from the unknown species may result in erroneous classifications, with high levels of confidence. This same scenario also applies to discriminant function analysis. It is therefore very important that the library of calls contain all species that may be encountered by the end user.

No classification system, particularly one that has been automated, can be left to run completely unattended. Automation of the system is designed to allow for the production of consistent, reproducible, objective (c.f. subjective) results, not necessarily to save time. In the case of my neural network-based system, each call is checked visually before and after call characteristics are extracted. The results of any classification must also be checked thoroughly for obvious (and sometimes not so obvious) mistakes. Particular attention must be paid to the confidence rating of each classification. To leave the system to run unattended and believe all the results it produces is simply bad science.

Before any signal is analyzed, researchers must realize that calls of different species of bat are not equally detectable. Some species produce extremely low amplitude calls and some species of gleaners do not use echolocation at all to localize prey. Other species of bat produce very high-amplitude calls. Low-amplitude calls may not be recorded, or recordings may not accurately represent the repertoire of a species. It should be noted, however, that the lack of a recording does not necessarily mean that the study animals are not echolocating.

The sensitivity of the microphone used, and its relative position to the bat emitting the echolocation calls, may mean that the calls are not sufficiently loud enough to elicit a response from the microphone. Recording techniques and situations can also affect the quality of signal recorded, and therefore our ability to identify the species emitting a particular call (Parsons and Obrist 2003; Parsons et al. 2000; Pye 1993).

Bats exhibit flexibility in the design of echolocation calls. Call structure varies with degree of acoustic clutter (e.g., Jensen and Miller 1999; Kalko and Schnitzler 1989, 1993; Rydell 1990; Simmons and Stein 1980), and at different stages in a bat's approach to objects such as prey items and roosts (Kalko 1995; Masters et al. 1991; Parsons et al. 1997; Simmons et al. 1979). Age also has an effect on the echolocation calls of bats, independent of morphology (Jones and Kokurewicz 1994; Jones et al. 1992; Masters et al. 1995). Morphology also influences call design and can cause convergence in call design between morphologically similar species (Bogdanowicz et al. 1999; Jones 1996, 1999).

Species identification systems must be able to cope with substantial intra- and interspecific variation in call design. They must also be able to separate species that produce seemingly identical calls. This is an enormously difficult task. Central to the development of such a system is the identification of call parameters that best separate species. Once identified, these parameters must be measured in a repeatable, quantitative manner to ensure the reliability of the results.

To classify calls to species level using a single analysis, given the number of species in this study and the number of variables measured per call, is not a trivial task, especially given the highly variable nature of the data. We hypothesized that the use of a hierarchical classification system, in which calls were classified to genus and species level by separate functions or networks, would improve the ability of the automated neural networks to correctly classify species by spreading the complexity of the task over several analyses. Ultimately, the success of such an approach is reflected in increased correct identification rates at the species level. At every level, (all species together, genus only, within genera containing multiple species) the automated neural networks outperformed their equivalent discriminant function analysis. Analysis uses a series of functions that best separate the groups and then classifies each data point in turn. However, the neural network we employed in this study used an error back-propagation algorithm (Rumelhart et al. 1986) based on the error-correction learning rule. Error back-propagation learning consists of two passes through the different layers of the network: a forward pass and a backward pass. In the forward pass, inputs are presented to the network and a signal passes through the various layers resulting in a set of actual response from the network. During the forward pass, the synaptic weights of the neurons in the network are fixed. During the backward pass, these synaptic weights are adjusted to make the actual response of the network match the desired response. The use of a network with hidden neurons i.e., neurons not part of the input or output layers, means that the network can learn complex tasks by extracting progressively more meaningful features from the input data. Therefore, given the complexity of species identification, it is not surprising that the artificial neural networks outperformed discriminant function analysis.

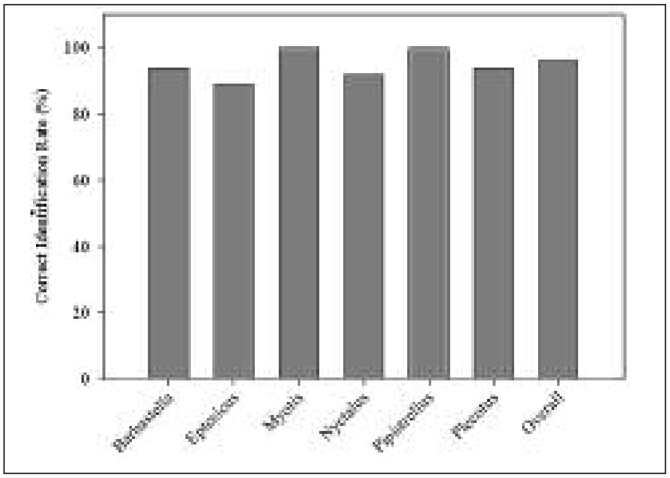

Fig. 5—Correct identification rates for the artificial neural networks trained to identify calls to genus level.

CONCLUSIONS

The accuracy and success of a species-identification system relies on the quality of the data presented to it and the conditions under which the recording was made (e.g., equipment used, degree of acoustic clutter). Parameters measured from calls must be chosen for low intraspecific and high interspecific variability. If possible, the entire echolocation repertoire of the bats should also be recorded. Using appropriate signal-processing techniques, parameters can be measured in a quantitative and repeatable manner. Classification systems must be flexible but also able to make subtle decisions when separating calls from different species. Artificial neural networks, because of the error-minimization routines, outperform traditional statistical methods.

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ADVANCED ANALYSIS TECHNIQUES FOR IDENTIFYING BAT SPECIES

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Despite the ready abundance of acoustic information that bats provide, confident acoustic species recognition for many bats has remained an elusive goal. Calls from bats that we think we know can be rendered uncertain when subsequent studies reveal an acoustically similar sympatric species capable of exhibiting a presumed discriminating characteristic within the time-frequency domain. Surmounting these challenges will require additional acoustic information beyond the time-frequency relationships on which many studies have concentrated. In addition to yielding additional acoustic information about call structure, acquiring full-spectrum acoustic data permits a complete evaluation of the acoustic environment, such as noise and other factors that can lead to spurious results if left unknown. As we build larger datasets of acoustic information and call libraries, we should expect to decipher more species-specific characteristics and apply them to the endeavor of identifying unknown bats from their vocalizations. With digital signal processing, we can combine various acoustic parameters of echolocation calls to produce transforms of time-varying interrelationships and represent them in two-dimensional plots. These plots can render striking visual contrasts between species with inexplicit distinctions in standard time-frequency plots.

Key words: acoustic, bat, echolocation, harmonics, sonar, SonoBat, spectrogram, species identification, Vespertilionidae
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INTRODUCTION

Discriminating bat species based on their vocalizations presumes that discernable differences exist. O'Farrell et al. (1999) asserted that species-specific characteristics exist, although revealing those differences may require the application of new technology. Barclay (1999) countered that bat species have no particular selective pressure to emit calls differently than any other species, and we should therefore not expect to find species-specific calls. Echolocating bats use their calls to serve a utility function to acquire information and we can expect that selection has acted to optimize that process.

It is thus unsurprising that many species with similar information needs have similar calls. Furthermore, to the dismay of many bat biologists, bats exercise considerable plasticity in their vocalizations and can produce call variants whose parameters overlap with those emitted by other species (Barclay 1999; Obrist 1995; Thomas et al. 1987). Bats that may emit different calls in low-clutter habitats may nonetheless exhibit similar calls in response to similar situations, e.g., among vegetation.

However reasonable Barclay's argument may be, the assertion made by O'Farrell and his colleagues cannot be entirely dismissed until we have exhausted all possible technological methodologies and innovations. If correct, Barclay's assertion will ultimately set some limit upon our capacity to identify bats, but I believe that the present state of the art still has considerable room to advance before reaching such a limit, if it exists. By continuing to apply new analytical methods to assess bat echolocation calls, we will undoubtedly reveal more of O'Farrell's suspected species-specific characteristics.

To proceed, we must presume that interspecific differences in morphology and foraging strategy should render differences in vocal signatures, however subtle. To use an analogy, under our current state of the art, we can readily discriminate bats speaking French from those speaking English, but we must next hone our tools to discriminate British accents from American and, in the most difficult of cases, a Philadelphia accent from that of a New Yorker. Discerning such subtleties entails evaluating a broader set of characteristics than just those required for distinguishing different languages.

Since Fenton and Bell (1981) provided the initial impetus, much of our interpretation of bat calls has focused upon their time-frequency character. While this has considerably advanced our understanding of bat vocalizations, it has nonetheless reached its limitation in more acoustically cryptic species. The time and frequency relationship provides a prescription not unlike a music score in which the notes designate the duration and frequency (pitch) of a song. For example, suppose that two musicians play the same song, but one plays it on a piano and the other a violin. A musical score interpretation from each would be indistinguishable if they played the same notes with the same tempo. However, even our non-Chiropteran ears could recognize which of the two instruments would be playing. The qualities that enable this discrimination are beyond the basic time and frequency information and include characteristics of amplitude variation and harmonics that enrich a sound signal. Full-spectrum processing can reveal this additional information content. Because bats emit vocalizations rich with amplitude variation and harmonics, we would be remiss to exclude such information in our efforts to reveal species-specific vocal signatures, particularly for bats that "score" similarly.

In this paper, I will present some further advantages of interpreting bat echolocation calls using full-spectrum sound analysis, and then present an alternative method of processing acoustic signals to improve the visualization of subtle differences between the echolocation calls of acoustically similar species.

PROCESSING AND INTERPRETING ACOUSTIC SIGNALS

Chris Corben's Anabat system and Zero-Crossing Analysis Interface Module (ZCAIM) (Titley Electronics, Ballina, NSW, Australia) can rapidly and efficiently produce simple time-frequency representations of bat vocalizations. These are generated from the frequency component of the signal with the strongest amplitude. In many situations, these basic time-frequency plots are sufficient to identify a number of bat species; more in the hands of an experienced operator. However, the efficiency of the Anabat system is gained at the loss of amplitude, harmonic, and additional frequency information that can benefit call discrimination and the search for additional species-specific characteristics. In addition to revealing additional information content of bat vocalizations, full-spectrum processing renders simultaneously occurring multiple-frequency information. This approach enables the identification of simultaneously occurring calls (Fig. 1), and also provides beneficial information about the overall acoustic environment in which the sound was recorded.

Noise happens, and you should know when it does

In field situations, we rarely acquire ideal recordings of calls. Atmospheric effects of wind and thermal convection, additional environmental sounds, echoes from clutter, and hardware artifacts all contribute to signal degradation. Full-spectrum sound processing renders all such frequency content visible. Simple visual inspection of full-spectrum spectrograms can be used to quickly recognize unsuitable data (Fig. 2). With the complete acoustic information visible, we can often interpret noisy data and discern the trend of a call within the noise, and do so better than using automated selection. Creating a reliable algorithm to duplicate our own judgment of the call structure within the noise remains a challenge. This situation will likely improve with advances in computing technology. Until then, we must rely on our own faculties for quality control of acoustic data.

Unfortunately, other effects can lead to spurious call processing. Even in apparently clean acoustic environments, echoes produced from a bat's own calls can extend and obscure the apparent ending of a call (Figs. 3 & 4). In a simple time-frequency representation, such effects may remain hidden to the user. With full-spectrum processing, they become readily visible and usable data can often be extracted from such recordings by interpreting the complete suite of acoustic information (see harmonic discussion below).

Sound dissipates

The orientation and distance of the bat from the recording microphone profoundly influences the quality of the signal received. It is imperative to recognize and reject data from bats that were simply too far from the microphone to render definitive signals for identification (Fig. 5). Such calls can appear clean, but may nonetheless lack characteristics essential for classification. In the worst cases, call fragments may mimic other candidate species.

The utility of harmonics

Except for those bats that switch power among their harmonics (e.g., *Macrotus californicus*), rendering harmonic content may seem superfluous to interpreting call structure. However, harmonics help solve some of the pitfalls described in the preceding paragraphs and afford some other assistance:

1) Because additional harmonics are typically lower in amplitude than the fundamental, their presence indicates a quality recording from a closely approaching bat. Thus, such recordings ensure that the call is not a fragment (Fig. 5) and represent good specimen calls.

2) Similarly, harmonics can be used to indicate the actual time at which a call ends, even when the fundamental may be obscured in noise (Fig. 4).

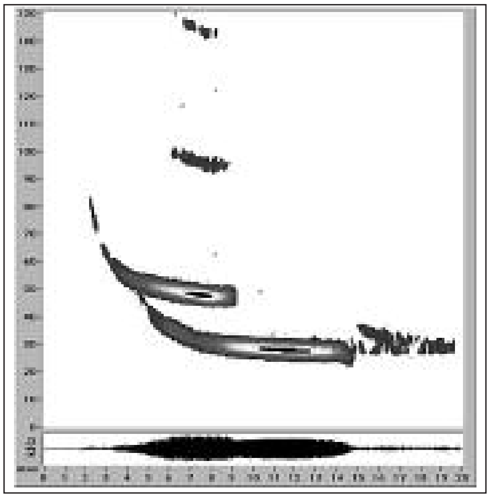

Figure 1: Simultaneously occurring calls from a *Pipistrellus hesperus* (top) and *Eptesicus fuscus* (bottom). The ability of full-spectrum data to render multiple frequencies at each point in time enables each of these bats to be identified.

Figure 2: Two calls from a western red bat, *Lasiurus blossevillii*. Noise from atmospheric effects and clutter echoes are clearly visible on the left call, compared with the clean signal on the right. The calls in this and all other figures were recorded and processed using SonoBat software (DNDesign, *www.sonobat.com*) and Pettersson D-240x and D-980 detectors.

3) By their nature, changes in slope are more pronounced in harmonics, and this can be exploited to interpret subtleties of call morphology. Harmonics occur as simple multiples of the fundamental. Two points in time on the fundamental at say, 42 and 40 kHz, will correspond to points at 84 and 80 kHz on the second harmonic, 126 and 120 kHz on the third harmonic, and so on. Thus the slope on the second harmonic between these time points will be steeper than the fundamental, and the third harmonic will be steeper than the second. For example, the calls from the western red bat, *Lasiurus blossevillii*, can be distinguished from *Myotis* species operating in the same frequency range by an upturn at the end of the call, as opposed to a downturn for *Myotis*. Such details are accentuated in upper harmonics (Figs. 2 and 4).

A cautionary note on interpreting harmonics: Harmonic artifacts can be produced by a process known as "clipping" in which the sound amplitude exceeds the upper limit of any step in the recording process. To perform a basic check for clipping-effect artifacts, inspect the amplitude envelope of the signal's oscillogram trace. The amplitude envelope of signals with clipping appear flat topped and featureless. Turn off any filters or other digital processing effects when making this inspection.

TAKE YOUR BAT CALLS TO THE LIBRARY

Effective species recognition based on bat echolocation calls depends upon knowing each species' acoustic characteristics. Because bats display considerable plasticity in their call structure (Barclay 1999; Obrist 1995; Thomas et al. 1987), species-specific characteristics must be defined as ranges rather than specific values. Furthermore, many sympatric species can have overlapping characteristics. However, by increasing the number of parameters used to characterize a call, the likelihood of uncovering discriminating characteristics also increases, along with the confidence in the discrimination analysis. Call libraries provide a vital resource for assembling, accessing, and interpreting species-specific characteristics, and provide comparative data for confirming species identifications.

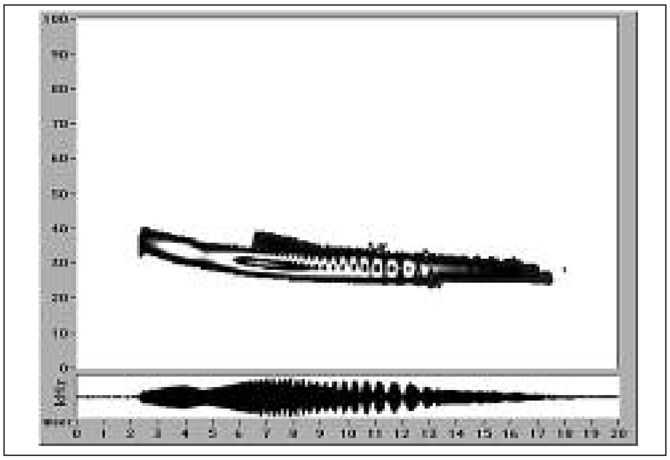

Figure 3: Search-phase call from a free-tailed bat, *Tadarida brasiliensis*, showing distortion patterns due to constructive and destructive interference from the overlap of the primary call signal and its echo. The pattern of beats in the oscillogram and sonogram would not be visible in a simple time-frequency rendering of this information. Furthermore, such processing would likely interpret this call to be 15 ms in duration, when in fact it is only 11 ms long.

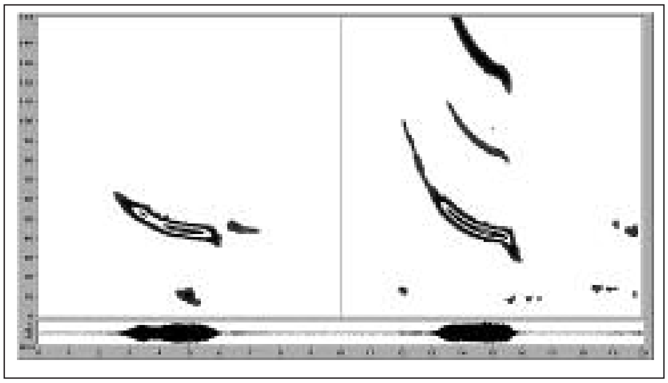

Figure 5: Calls from a western small-footed myotis, *Myotis ciliolabrum*, flying nearly out of range (left) and in close proximity (right) to the recording microphone. Although the call on the left lacks noise, it is nonetheless a fragment lacking the complete characteristics needed for positive species identification. Most bats vary amplitude (loudness) within their calls, and the lower amplitude portions will not be recorded from bats flying farther from the microphone. However, for some bats, the low-amplitude call portions may contain vital diagnostic characteristics. It is incumbent on the investigator to know if the candidate species do, and seek them as assurance for confident species recognition, particularly for presence/absence-type surveys.

Note: *The amplitude mapping shown by the contours only indicates the relative amplitude distribution within each call and does not show the relative amplitude between the two calls. Although different in absolute amplitude relative to the recording microphone, the amplitude in these calls was normalized to the same values for display.*

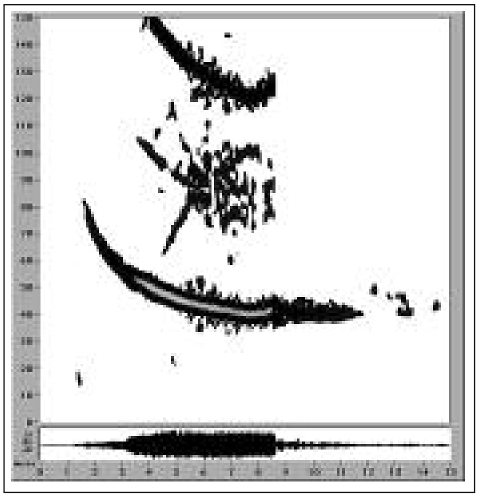

Figure 4: Search-phase call from a western red bat, *Lasiurus blossevillii*, showing echo noise obscuring the ending details of the fundamental. The second harmonic is obscured in noise, but the third harmonic is relatively clear and can be used as a reference to the end of this call. Using the onset of the fundamental to the end of the third harmonic reveals this call to be about 7 ms in duration, whereas an interpretation of the call duration based solely on the fundamental and its trailing noise would interpret this call to be as long as 12 ms. In addition, the upturn at the end of the call, a diagnostic feature of the western red bat, is accentuated and only clearly visible in the third harmonic, a feature that would be missed if only the dominant amplitude portion of the signal was rendered.

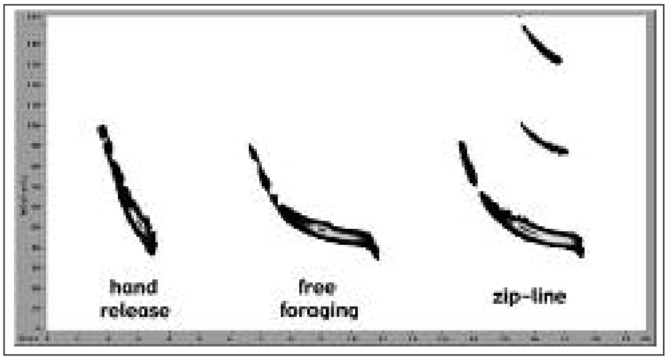

Figure 6: Comparison of *Myotis ciliolabrum* calls recorded from a hand-released bat, a free-foraging bat, and a bat flying on a tethered zip line. The hand-released call shares characteristics with calls emitted by this species when flying in clutter, even though it was released in an open meadow. Note the morphological similarity of the free-flying call with that of the zip-line call. The zip-line recording exhibits stronger harmonics because of the close proximity to the detector.

Know when to say 'No'

Within a geographic area, the overlapping parameters of sympatric species should be recognized, and the species-specific ranges of these parameters should be organized into "discriminating subsets" on which confident species recognition can be based. Unknown calls that fall outside the discriminating subsets should be rejected.

Building call libraries

Search-phase calls from free-flying bats are the most consistent and species-discriminant call variety (Betts 1998) and are the variety most often acquired from unknown bats in the field. The challenge of assembling a reference library lies in acquiring calls of this type and knowing the habitat and species of bat that produced them. A combination of methods may be used to acquire and match bats with recorded calls to assemble or contribute to a reference call library. Most often used are:

1) calls recorded from captured bats released by hand
2) calls recorded from bats flying near known roosts
3) calls recorded from free-flying bats from which a visual identification can be made with the aid of a spotlight or night-vision equipment
4) calls recorded from light-tagged free-flying bats (Hovorka et al. 1996)
5) calls recorded from captured bats flying tethered to a zip line (Szewczak 2000a).

Bats flying in closed spaces or immediately after taking flight from a roost or after a hand release do not consistently emit calls representative of their free-flying search-phase calls. The bats are accelerating, orienting, and not in a steady mode of flight (Betts 1998). For this reason, calls recorded from hand releases of positively identified captured bats seldom yield suitable results (Fig. 6). Satisfactory calls can be acquired from bats flying near roosts of known species composition, but care must be exercised to acquire calls from bats leaving the roost after they fall into a routine foraging mode. Visual identification can be particularly helpful for bats that avoid capture, or are flying in situations that make capture difficult. Light-tagging entails temporarily attaching a mini-cyalume light stick to a captured bat that is then released. With luck, a light-tagged bat will later be recorded as it passes. Different species captured during a session can be fitted with different color light tags. Light-tagging is perhaps the best method for acquiring standard reference calls because the recordings are acquired from bats foraging naturally. However, the acoustic recovery rate of light-tagged bats is often disappointingly low. Most light-tagged bats fly off, never to be seen again, or if they are seen, they are often too far out of detector range to render suitable specimen calls (Fig. 5).

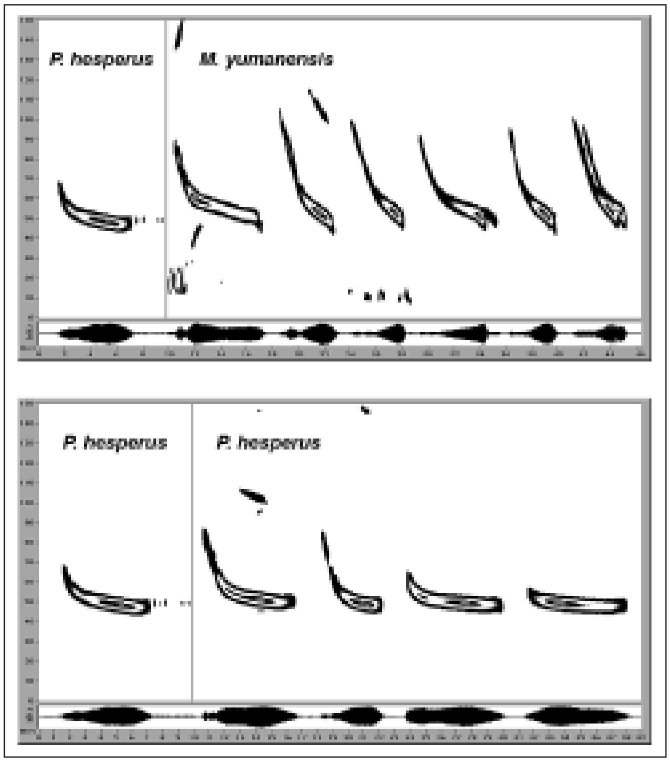

Figure 7: Top panel: spectrogram display of a single *Pipistrellus hesperus* call (left) compared against a compilation of *Myotis yumanensis* library reference calls (right) displayed at the same scale. Bottom panel: the same *P. hesperus* call compared against a compilation of *P. hesperus* library reference calls displayed at the same scale. These compilations were assembled from different bats recorded at various locations. Compiled reference views can display a bat's full call repertoire at a glance and greatly facilitate comparative species discrimination. Note the conserved characteristics across the compilations.

The frustratingly low recovery of light-tagged bats prompted the development of the tethered zip-line method for acquiring reference calls. Captured bats are attached to a 2-m length of small elastic cord (elastic sewing thread) by a loose-fitting loop of the cord pulled over the bat's head. The other end of the elastic cord is attached via a small snap swivel to 30–50 m of taut monofilament line about 1 m above the ground. A light-tag is hung on the zip line such that the snap swivel pulls it along, which aids in tracking the bat. The zip line is readily deployed in the field near the capture site using two poles or other fixed objects. The length of the zip line enables bats to achieve a steady mode of flight before recording calls. Many individuals provide good flights along the zip line on their first try. Some need

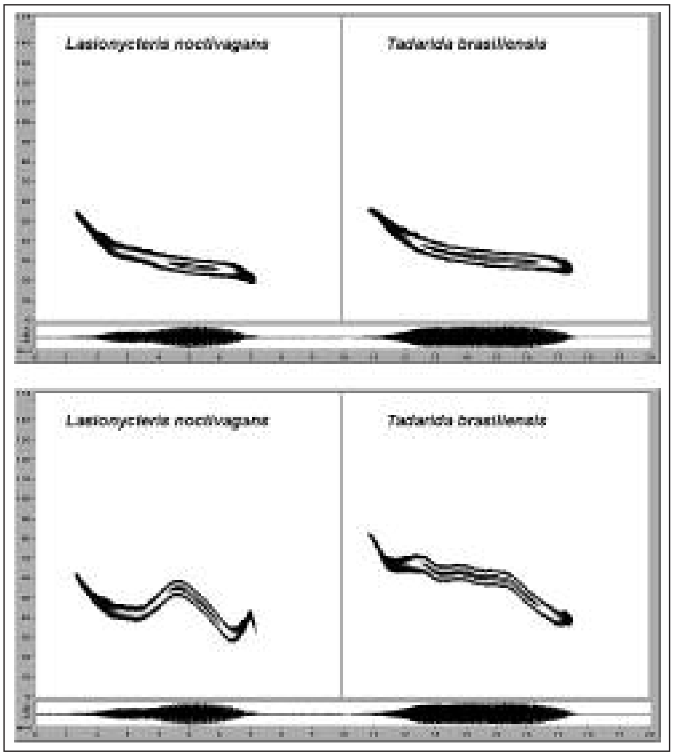

Figure 8: High-resolution comparative views of calls from *Lasiurus noctivagans* and *Tadarida brasiliensis* indicating similar time-frequency characteristics when displayed on a standard time-frequency spectrogram (top panel). Some differences are visible in the amplitude as shown by the gray-scale contours. However, those differences are rendered strikingly different when the same calls were reprocessed using a power-slope transform in which the vertical position of the spectrogram is changed as a function of the slope and amplitude relationship throughout the call (lower panel).

several attempts before achieving steady flight. A primary advantage of the zip-line tether method is that it provides the opportunity for repeated flights to record satisfactory calls.

Zip lining can produce representative recordings of calls (Fig. 6). However, it does not work for all bats in all situations. A combination of the methods listed above may be used to ensure the completion of a thorough and representative call database, and the acquisition of multiple samples from species across a particular study region to fully characterize their call repertoires.

Sampling from the library

In addition to self-constructed call libraries, reference calls may be acquired through Internet-based call libraries. To date, most of these have compiled Anabat format calls. However, with the increasing popularity of full-spectrum call recording, more data in this format will become available. One example is a recently opened web-based call library specializing in full-spectrum call data at *www.batcalls.org*. (And I encourage everyone collecting full-spectrum recordings of bat calls to contribute to this resource.)

From self-constructed and centralized call libraries, discriminating subsets of species' call repertoires may be assembled for extracting quantitative information for the interpretation of unknown calls. Alternatively, the call files can be used for direct comparison with unknown calls. SonoBat software allows selected reference calls to be assembled into compilations that facilitate the rapid comparison of an unknown call with that of a species' call repertoire (Fig. 7).

Translating Sound and the Analysis of Bat Echolocation Calls

In the search for species-specific characteristics to identify bats by their echolocation calls, the analysis should embrace a robust set of call characteristics. Numerous parameters have been used to describe and measure call structure and sequences (e.g., Betts 1998; Fenton and Bell 1981; Obrist 1988; Oliveira 1998; Parsons and Jones 2000; Szewczak 2000b). Quantitative approaches applied to call analysis have ranged from simple descriptive comparisons (MacDonald et al. 1994; O'Farrell et al. 1999) to more sophisticated approaches employing discriminant function analysis (Krusic and Neefus 1996; Lance et al. 1996; Parsons and Jones 2000) and artificial neural networks (Parsons 2000; Parsons and Jones 2000). The latter quantitative approaches demonstrate the usefulness of incorporating characteristics beyond those that can be derived just from the time-frequency information, e.g., the frequency of maximum power. While quantitative approaches hold promise for eventual automation of species recognition, for now it remains productive to seek species-specific characteristics through visual inspection of spectrogram representations of acoustic recordings. As powerful as computers have become for processing information, they have yet to replace the impressive visual processing capacity of the human brain (Tufte 1983).

Seeing the differences

Discriminating calls with similar time-frequency characteristics is a frustrating challenge. We can show our students numerous examples and belabor the subtle details that enable species recognition, but in the end many will proclaim, "But they still look the same to me!" However, it is possible to graphically display transforms of the standard time-frequency spectrogram plots that incorporate characteristics beyond the basic time-frequency information, e.g., the relationship of the change in amplitude per time with the change in frequency per time, i.e., slope (Fig. 8). For example, qualities that have proven species-specific through discriminant function or artificial neural network analysis can be made visible and used to enable perception of emergent patterns among different species. In addition, it is satisfying to "see" the differences revealed through abstract analytical processes.

As an example of this approach, I applied three different transforms to calls from three sympatric species with calls having similar time-frequency characteristics: the California myotis (*Myotis californicus*), the Yuma myotis, (*M. yumanensis*), and the western pipistrelle (*Pipistrellus hesperus*). Simple time-frequency plots of the dominant frequency information show the particular similarity of the two *Myotis* spp. calls (Fig. 9, top row). When processed as full-spectrum spectrograms, some variation in the amplitude distribution becomes visible between them, but the pipistrelle is revealed to have a similar amplitude distribution as the California myotis (Fig. 9, second row). However, a power-slope transform in which the vertical position of the spectrogram is changed as a function of the interrelationship between slope and amplitude throughout the call accentuates the differences suggested by the full-spectrum spectrogram in the amplitude distribution between the *Myotis* spp. (Fig. 9, third row). However, consistent with the similarity in the amplitude distribution between the California myotis and the pipistrelle (Fig. 9, second row), their power-slope transforms remain similar. But a transform in which the vertical position of the spectrogram is changed as a function of slope (with an amplitude normalization) reveals a clear distinction between the two species (Fig. 9, bottom row). Eventually, we may reveal sets of transforms that facilitate routine discrimination of presently difficult species, and we will have a way to show that all species can "look" different.

ACKNOWLEDGEMENTS

I thank Michelle McKenzie and Ted Weller of the U.S. Forest Service Sierra Framework Bat Monitoring Team for contributing the *Lasiurus blossevillii* calls.

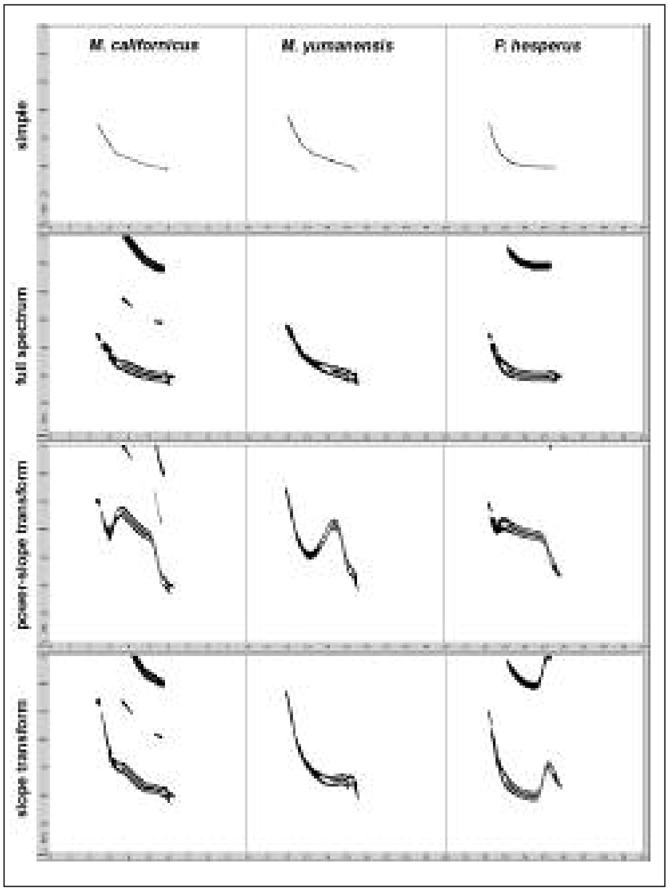

Figure 9: Calls from *Myotis californicus*, *M. yumanensis*, and *Pipistrellus hesperus* displayed as simple time-frequency plots, full-spectrum spectrograms, power-slope transforms, and slope transforms. In the power-slope transform, the vertical position of the spectrogram is changed as a function of the interrelationship between slope and amplitude, and in the slope transform the vertical position of the spectrogram is changed as a function of the slope throughout the call. Note how the transform plots reveal distinct visual differences compared with the time-frequency characteristics displayed in the top row.

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WRAP-UP: USING BAT DETECTORS FOR SPECIES IDENTIFICATION

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The use of different study designs, detection systems, and analysis techniques depends on various factors:

1) **Cost.** At times, there is a trade-off between cost, personnel, and data requirements (e.g., download time and the cost in lost time between frequency division and time expansion).

2) **Geographic Scale.** Intensive in a small area or an area that you can move from habitat to habitat quickly versus a large area where transects and sampling in person are not possible.

3) **Temporal Scale.** Is this a 2-week survey to determine what species are present or a multiyear project?

4) **The Question.** Are you interested in the presence or absence in specific locations or relative activity levels in different habitats for which you want multiple examples or replicates?

Numbers 2 and 3 above partly explain the difference in approach between European and North American bat-detector studies. We have to recognize that no matter how good the call library or observational data, not all bat passes will be identifiable to species. And not all species are equally identifiable (e.g., the "dreaded *Myotis*" and the "evil *Plecotus*").

Training personnel in interpreting calls is vital, no matter what system is being used or what question is being asked. It is unrealistic to expect to get the detecting system one day and be using it to collect species-specific data the next. Not all field personnel are equal in their abilities, just as not all detectors, even within a single type, are equal. Therefore, it is crucial to calibrate personnel and equipment and to randomize their deployment.

Purely statistical analysis of call structure will often not be as successful at identifying bats to species as it will be to incorporate in-person, behavioral observations. Likewise, call repetition rate, as well as its variation, is an important and useful variable. There are a number of different analysis techniques. For example, neural networks generally identify species better (i.e., out perform) than discriminate function analysis.

Finally, a call library is most useful when it includes the entire repertoire of a species. Flight-cage studies may be useful for some purposes to get parts of some species' call repertoires. The use of social calls may also facilitate species identification.

Section 4

RESOURCES, RESEARCH, AND STUDY USING BAT DETECTORS

FUTURE DIRECTIONS

BAT-SPECIFIC SIGNAL ANALYSIS SOFTWARE: BATSOUND

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BatSound is Windows-based software useful for recording, playback, and analysis of bat calls transformed by bat detectors. It offers various analysis methods, such as computation of spectrograms, oscillograms, and power spectra. BatSound Pro also supports the use of high-speed data-acquisition cards, enabling recording and playback of ultrasonic signals without the need of first transforming them using a bat detector.

Key words: BatSound, power spectrum, real-time spectrogram, sound analysis software, ultrasound playback, ultrasound recording

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INTRODUCTION

A variety of computer programs are available for analysis of sound, although few are specifically designed for recording and analyzing ultrasound. Ultrasonic signals can be studied using a computer either by first transforming the high-frequency signals into audible ones, using a bat detector and then feeding the signals to the computer's audio sound input, or by directly recording the bat call into the computer through a high-speed data acquisition card. After capturing the calls, they can be analyzed in a variety of ways.

BatSound (Pettersson Elektronik, Uppsala, Sweden) is Windows-based software for recording and analyzing bat calls. It comprises a number of general sound analysis tools as well as some echolocation call-specific functions.

The purpose of this paper is to provide an overview of the most important functions of BatSound, followed by a description of some of these.

OVERVIEW

In order to take full advantage of the different analysis modes in BatSound, time-expanded signals or signals obtained by high-speed recording need to be used.

BatSound displays the signals in different forms, such as an oscillogram, a spectrogram, or a power spectrum. While the signal is being recorded, the spectrogram and/or oscillogram are shown in real time on the screen, which is useful for immediate evaluation of the recorded signal. Recorded signals can also be edited in many ways. For example, they can be digitally filtered to improve the sound quality. The output diagrams can be printed or copied to other applications.

BatSound has an automatic parameter-extraction feature, which calculates call parameters, such as maximum or minimum frequency, pulse duration, etc. The analysis results can be copied to applications such as spreadsheet programs.

BatSound has a number of ultrasound-specific functions, such as automatic compensation of diagram scale values for any chosen time expansion factor. The "Virtual Bat Detector" is another special operating mode in which time-expanded recordings are played back as heard through a heterodyne bat detector, which is useful for those learning how to use or improve their skills with detectors.

The "Pro" version of BatSound supports high-speed sound cards that allow direct recording and playback of ultrasonic signals without first transforming the ultrasonic call using a bat detector. Both the standard and "Pro" versions use the regular audio sound card of the computer for recording and replay of audible signals, i.e., bat calls transformed with a bat detector.

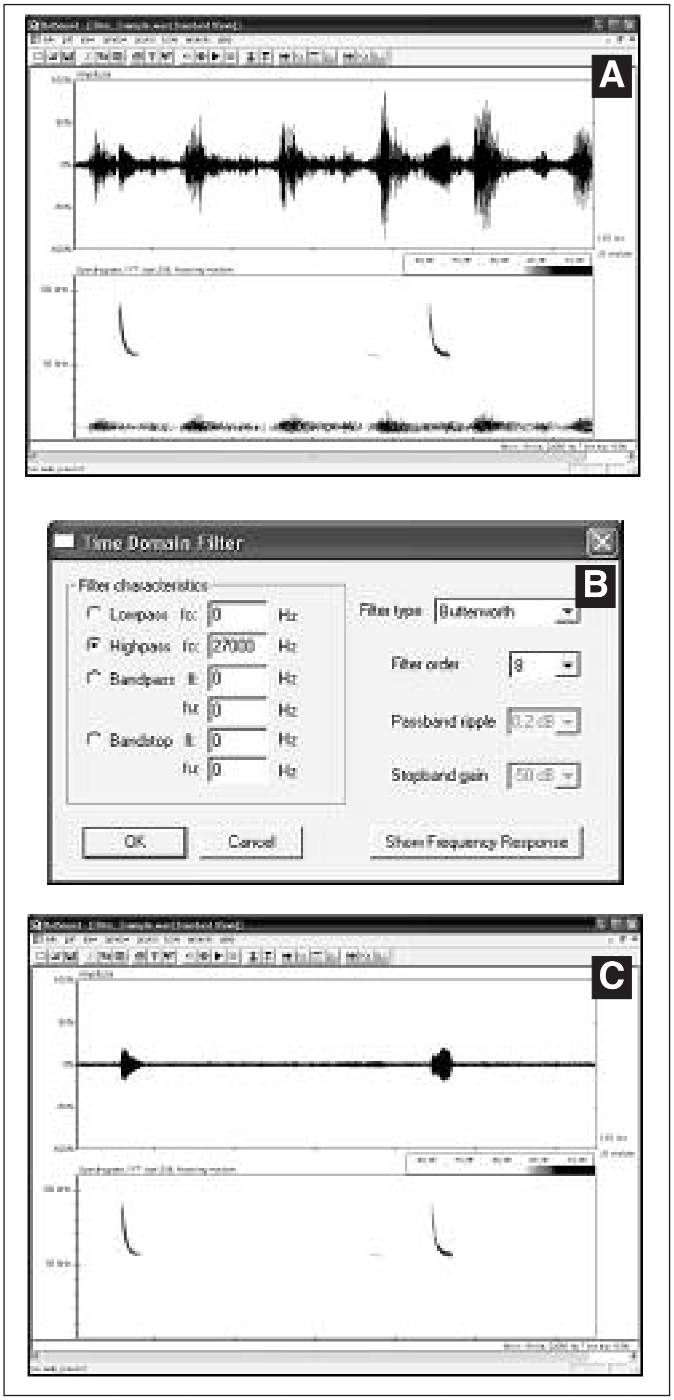
TYPICAL RECORDING AND ANALYSIS SESSION

Assuming that time-expanded bat echolocation calls have been recorded on tape, the following describes how to analyze these calls using BatSound. If the output of the tape recorder has been connected to the input of the computer's sound card and configured properly, BatSound is ready to make recordings.

First, the recording format must be chosen (8- or 16-bit resolution, sampling frequency). If automatic correction of the time and frequency axes values is desired, the time expansion factor should also be entered in the Sound Format dialog box. Selecting the "Record Sound" command or hitting the Record button in the toolbar of the BatSound window will start the recording, and the signal's spectrogram and/or oscillogram is displayed in real time for as long as the recording goes on. It is possible to make one single recording of the entire tape or to generate a number of recordings to separate files. In the former case, the file can be visually inspected and the interesting parts saved to separate files.

Once calls have been saved in a file, they are available for editing, playback, or further analysis. Fig. 1 shows how low-frequency noise can be eliminated by filtering.

There are a number of different spectrogram- and oscillogram-analysis parameters that can be used to change the appearance of the output. For example, the color mapping of the spectrogram can easily be changed. The mapping can be chosen from a set of predefined



Time Domain Filter

Filter characteristics

○ Lowpass fc: 0 Hz

● Highpass fc: 27000 Hz

○ Bandpass fl: 0 Hz

fu: 0 Hz

○ Bandstop fl: 0 Hz

fu: 0 Hz

Filter type: Butterworth

Filter order: 8

Passband ripple: 0.2 dB

Stopband gain: -50 dB

Figure 1: The oscillogram and spectrogram of ultrasonic signals with severe interference from low-frequency noise (mainly human voices) also present. Using a high-pass filter, the low-frequency noise can easily be eliminated by filtering. ***A:*** Original signal; **B:** Filter settings; **C:** Filtered signal.

mappings or designed freely by the user (Fig. 2).

A spectrogram, which is composed of a large number of power spectra partially overlapping in time, allows spectral changes over time to be studied. However, sometimes it is valuable to view the power spectrum over a specific time interval only. This function can be facilitated using the Power Spectrum command (Fig. 3).

There are also a number of temporal analysis modes in BatSound, e.g., to automatically calculate pulse length (duration) and interpulse interval (Fig. 4).

The data extracted from the different analyses can be used to assist in species identification. Obviously, knowledge of the sound characteristics of different species is required in order to accomplish this successfully.

FEATURE DETAILS

Automatic parameter calculation

To quickly process large files, an automatic parameter-calculation function is available. This enables important signal parameters such as signal duration and minimum and maximum frequencies, to be calculated (Fig. 5).

I emphasize that the results of any automatic-analysis function must be interpreted with caution. The program will not always make the same decisions that would be obtained by manually analyzing signals. In order for the user to obtain visual information about the results of the analysis, it is possible to have the program mark the different positions in the diagrams (pulse start/end time, maximum/minimum frequency).

Automatic recording mode

In the automatic recording mode, the computer is

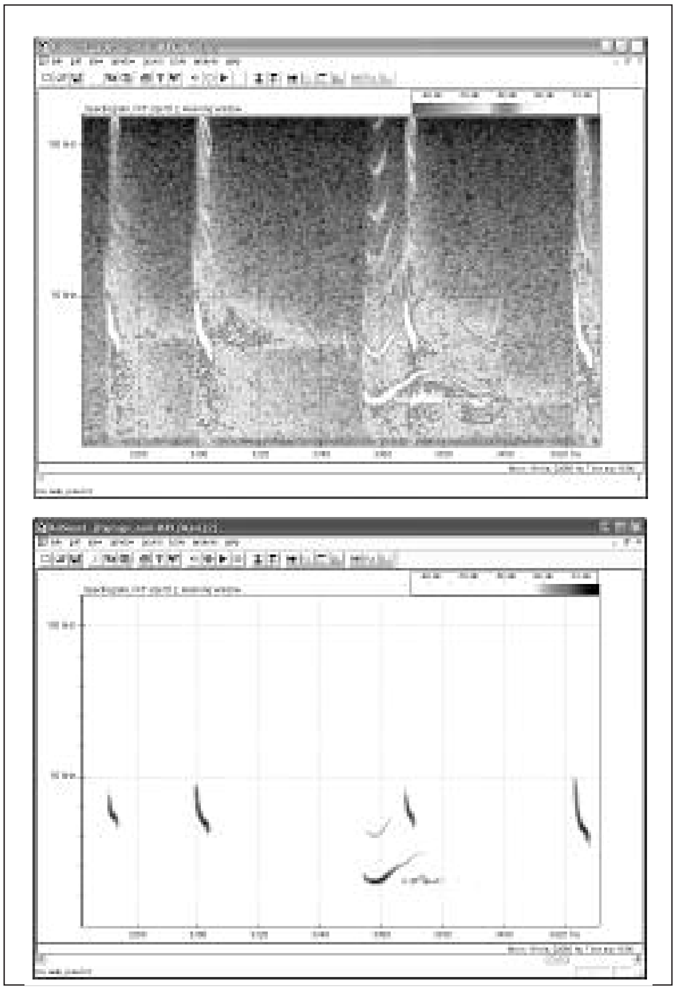


Figure 2: Different color mappings in a spectrogram (show in black and white).

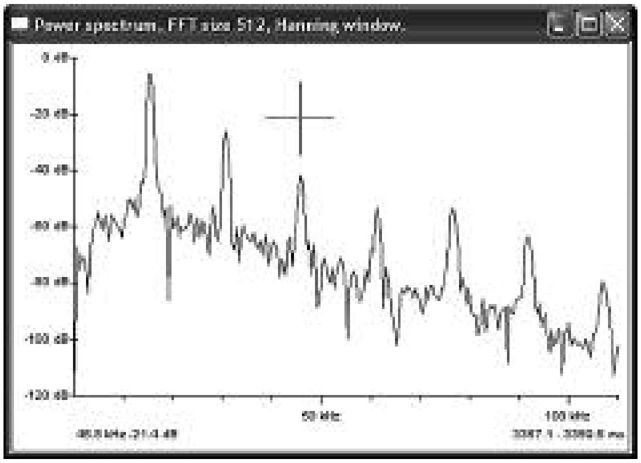


Figure 3: A power spectrum. The coordinates for the cursor are shown in the bottom left part of the diagram.

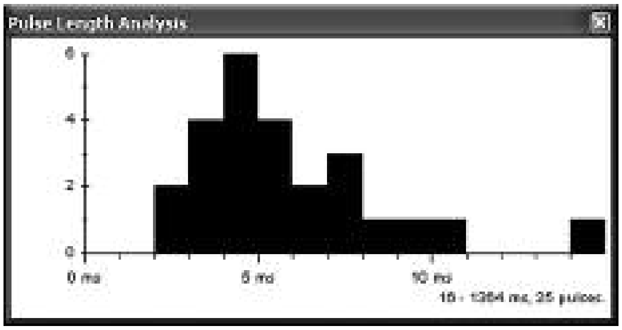


Figure 4: The distribution of pulse durations.

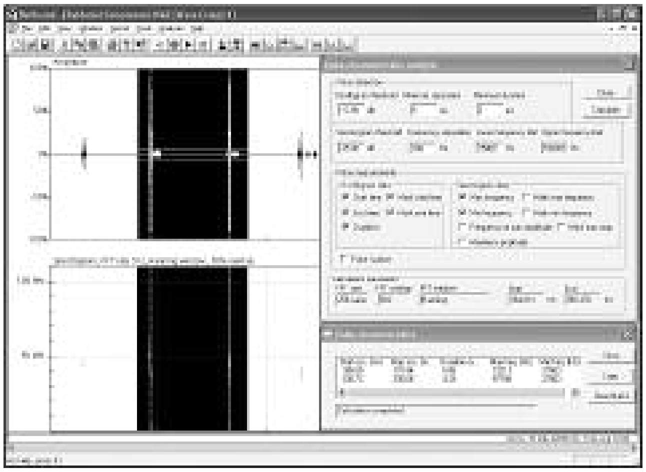
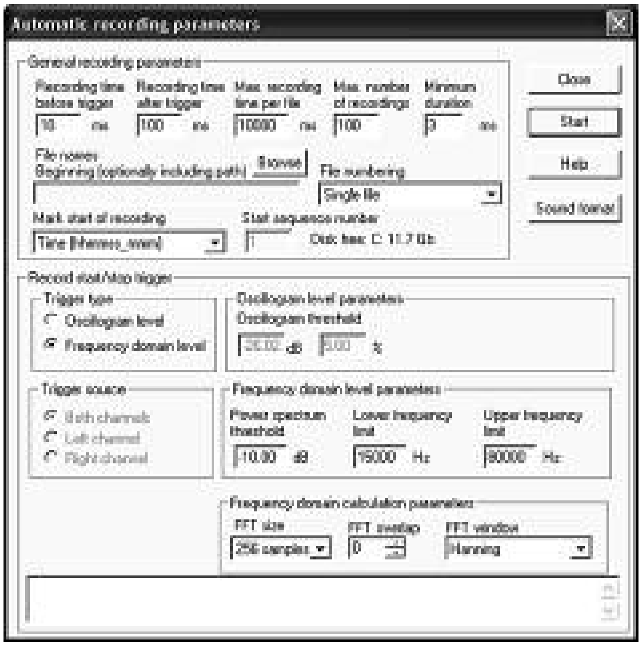


Figure 5: Automatic calculation of pulse characteristics. The analysis setup and result window are shown on the right.

essentially turned into a versatile "voice activated recorder" that starts recording once certain triggering conditions are met. The first requirement to make the program start recording is that the signal exceeds a minimum threshold level. This can also be made in a frequency selected mode, which requires that the signal also be within a certain frequency range to trigger the recording mechanism. The user can choose whether to make different recordings one after the other in one single file or to use a new file for each recording. A time stamp for each recording can be added if desired. Many other triggering parameters can be set (Fig. 6).



Automatic recording parameters

General recording parameters

Recording time before trigger: 10 ms

Recording time after trigger: 100 ms

Max. recording time per file: 10000 ms

Max. number of recordings: 100

Minimum duration: 3 ms

File names

Beginning (optionally including path):

File numbering: Single file

Mark start of recording: Time (hhmmss_mmm)

Start sequence number: 1

Disk free: C: 11.7 Gb

Record start/stop trigger

Trigger type

○ Oscillogram level

● Frequency domain level

Oscillogram level parameters

Oscillogram threshold: -26.02 dB 5.00 %

Trigger source

● Both channels

○ Left channel

○ Right channel

Frequency domain level parameters

Power spectrum threshold: -10.00 dB

Lower frequency limit: 15000 Hz

Upper frequency limit: 80000 Hz

Frequency domain calculation parameters

FFT size: 256 samples

FFT overlap: 0

FFT window: Hanning

Figure 6: The automatic recording parameters dialog.

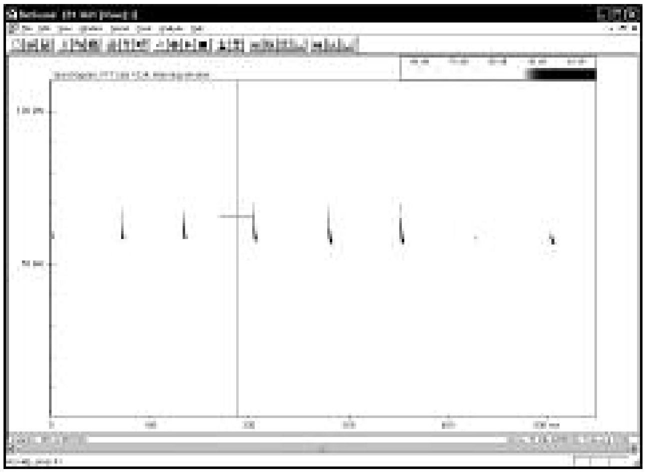


Figure 7: The spectrogram in the Virtual Bat Detector mode. The horizontal line on the vertical cursor cross indicates the tuned frequency. In the lower left corner, the actual time and the tuned frequency are shown.

Virtual Bat Detector

In the Virtual Bat Detector mode, time-expanded bat calls are replayed just as they would be heard through a heterodyne bat detector. An on-screen tuning control allows the user to adjust the tuned frequency as the sound is being replayed (Fig. 7). This mode is useful for beginners to learn how to identify different species using a heterodyne detector. It is informative to see the tuning control in the spectrogram of the signal at the same time as the bat calls are heard. Using this mode allows an understanding of the relation between certain pulse shapes and the resulting output from a heterodyne detector.

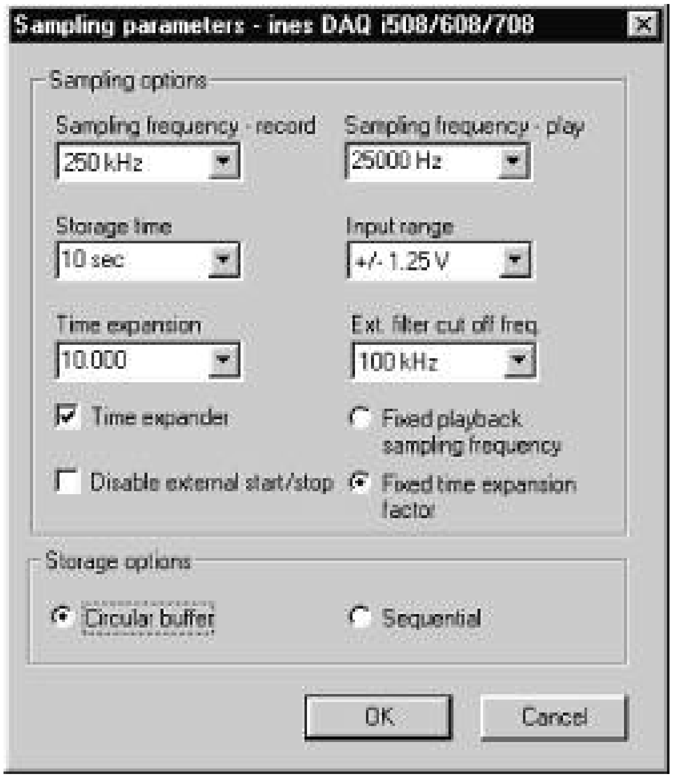
BATSOUND PRO: THE HIGH-SPEED MODE

Using the high-speed mode, which is only available in BatSound Pro, sampling rates of up to 500 kHz can be used. This theoretically makes it possible to record and replay signals up to 250 kHz (realistically up to about 200 kHz). This means that ultrasonic signals can be recorded without first being transformed using a bat detector. This mode requires a separate high-speed data acquisition card. In order to replay signals in this mode, the high-speed card should have a D/A converter. All supported cards are of the PCMCIA (PC-CARD) type, so laptop computers can be used, resulting in a compact unit.

Compared with using a time-expansion bat detector, the high-speed mode of BatSound Pro provides higher sound quality, since only one A/D conversion has to be made. The supported high-speed cards also use 12-bit A/D converters compared with the 8-bit converters commonly encountered in bat detectors. Another advantage is that the high-speed mode makes it feasible to make much longer recordings than a time-expansion bat detector would allow.

Using BatSound Pro allows recording in the high-speed mode to be done without accessing the keyboard of the computer. The recording process can be controlled from external switches, so the computer can be carried in a backpack. If desired, it is also possible to control the recordings via the mouse or keyboard. A separate control unit, the F2000 (Pettersson Elektronik, Uppsala, Sweden), is available to facilitate making high-speed recordings. This unit contains a switch to start and stop the recordings, an amplifier, and a lowpass filter with a selectable cut-off frequency.

The high-speed recording can be made either in a sequential or a circular buffer storage mode. Different sampling frequencies and recording times can be chosen (Fig. 8). At the option of the user, the program can be made to replay the recorded sound in a time-expanded form, which effectively turns the computer into a time-expansion detector.


Sampling parameters - ines DAQ i508/608/708

Sampling options

Sampling frequency - record: 250 kHz

Sampling frequency - play: 25000 Hz

Storage time: 10 sec

Input range: +/- 1.25 V

Time expansion: 10.000

Ext. filter cut off freq.: 100 kHz

☑ Time expander

☐ Disable external start/stop

○ Fixed playback sampling frequency

● Fixed time expansion factor

Storage options

● Circular buffer

○ Sequential

Figure 8: The “Sampling parameters” dialog box in the high-speed mode.

REPORTING: ESSENTIAL INFORMATION AND ANALYSIS

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Studies of the echolocation behavior and calls of bats may use data about calls as indicators of distribution and patterns of habitat use (means to an end) or as the end itself (studies of echolocation and calls). Research questions that are the focus of a project dictate the nature of data, equipment (hardware and software), and personnel required for work. The questions also influence the design of the data collection schedule, selection of sites, and statistical analyses. In studies of bat activity, the fundamental unit of data is the train of echolocation calls recorded or detected as a bat passes through the airspace sampled by the microphone (a bat pass). In studies of variation in echolocation calls, units of data are those used to describe the calls and their patterns of emission. When the focus is echolocation behavior, the data are those used to describe the calls, as well as those describing the behavior of the bats. The degree of complication (for data, equipment, personnel, and analysis) of any of these studies is determined by the numbers of species involved and the degree of similarity between their echolocation calls. In many studies of activity, tunable bat detectors will suffice, while work involving more detailed information about echolocation calls requires broadband bat detectors and more elaborate and detailed analysis of calls.

Key words: bat detectors, bat pass, Chiroptera, echolocation, fast Fourier transform power spectrum

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INTRODUCTION

The sheer quantity of echolocation calls produced by bats makes it relatively easy to acquire lots of data. The decision about which data are important depends upon the questions that will be addressed with them. Decisions about data and questions determine the choice of hardware and/or software required for the project. Decisions about which question(s) to address should be informed by knowledge of the biology of the bats. For example, as reported by Griffin (1958) and many others, species producing low-intensity echolocation calls (60-80 dB sound pressure level [SPL] @ 10 cm) are much less detectable than those using high intensity echolocation calls (≥ 110 dB SPL @ 10 cm). In most situations, assessing the presence, behavior, and activity of bats using low-intensity echolocation calls cannot be achieved by monitoring their echolocation calls. One of the worst-case scenarios for using echolocation calls to study the behavior and ecology of bats is species such as *Myotis emarginatus* that alternate between using high-intensity and low-intensity echolocation calls (Schumm et al. 1991). *Myotis dasycneme* also produces calls of very different intensities, from lower-intensity calls when hunting near vegetation or over small pools, to intense calls when hunting over larger bodies of water (Limpens 2001).

Under most circumstances, data derived from echolocation calls provide information about populations rather than about individuals (Thomas and Taylor 1990). This is an important limitation for projects whose goals require information about the behavior of individuals. Where individual bats or groups of bats can be identified by their calls, monitoring vocalizations may provide data about individuals. To date, these appear to be the exceptions rather than the rule.

DISPLAYING DATA

When the output of a broadband detector has been recorded, the sequence of calls produced by a bat flying through the airspace sampled by the microphone can be displayed as changes in amplitude (signal strength) over time (Fig. 1a), or as changes in frequency over time (spectrogram or time-frequency display; Fig. 1b). The output of a bat detector tuned to one frequency will produce a signal whenever it detects energy at the appropriate frequency, preserving information about timing but not about duration or patterns of change in frequency over time.

Expanding the display of any one call (Fig. 2) permits closer examination of how calls change in frequency over time. Using fast Fourier transform to obtain power spectra (Fig. 3) allows accurate and repeatable measurements of frequency components. Changing the baseline (compare vertical axis in Fig. 3a and b) permits closer examination of the power spectra associated with the calls (Fig. 3b), as opposed to background noise (Fig. 3a).

Good-quality recordings have a high signal-to-noise ratio, meaning that the signal (s in Fig. 4) is strong relative to the background noise (n in Fig. 4). Changes in signal strength across the calls comprising a bat pass (Fig. 1) are typical, probably reflecting a combination of changes in the bat's position relative to the microphone, including head movements.

BAT ACTIVITY

When the goal of a project is to use echolocation calls to monitor the activity of one species of bat, the selection of equipment and the data required are relatively simple.

Ideally, the data will be recorded as "bat passes" where a pass is the train of pulses recorded from an individual as it passes through the airspace sampled by the microphone (Fig. 1). Bat passes obvious to an observer listening to the audible output of a bat detector may be much less conspicuous in a visual display because of the time involved. By watching the bats as you listen to the output of the bat detector, the distinction between different bat passes may be more obvious. The horizontal scale on a visual display of a minute of recording, for exam-

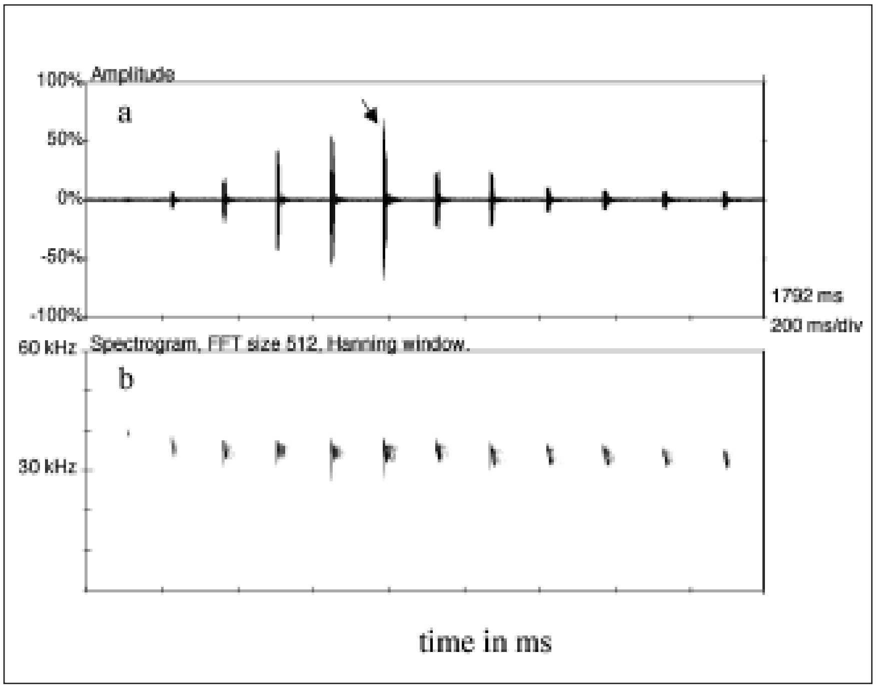

Figure 1: The sequence of calls produced by a *Molossus molossus* as it flew through the air space sampled by a Pettersson D980 bat detector. Shown are the (**a**) time-amplitude and (**b**) time-frequency displays. Note the consistent interpulse intervals reflecting synchronization of wingbeat and pulse production. The arrow identifies one of the calls shown in more detail in Figs. 2 and 3.

ple, will not show individual calls, which are just too short to register. Expanding the horizontal scale of the display to show individual calls can mask pass events.

When an individual flies back and forth within the airspace sampled by the microphone, the distinction between pass events may be clear (Fig. 1). Consistent interpulse intervals (Fig. 1) reflect the link between wingbeat cycle and pulse emission (Speakman and Racey 1991) and suggest that one individual produced the calls, but this is only for one pass. Often it is difficult to distinguish between separate "pass" events, particularly when interpulse intervals vary and there appears to be more than one bat active in the airspace sampled by the microphone. Outside a roost from which many bats emerge, the net effect can be a wall of sound offering no prospect of distinguishing between passes of individuals (Fig. 5).

Studying activity by monitoring echolocation calls can be achieved using a tunable bat detector and knowledge about the frequency dominating the species' echolocation calls. The literature may provide a general indication of what frequencies dominate the calls, and this can be adjusted according to local conditions (the bats, the detector, and the habitat). If there is only one species in the area, then watching the bat while tuning through the range of frequencies should allow the observer to hear the bat's calls. Earphones are often important, especially when several observers are listening to the output of different bat detectors.

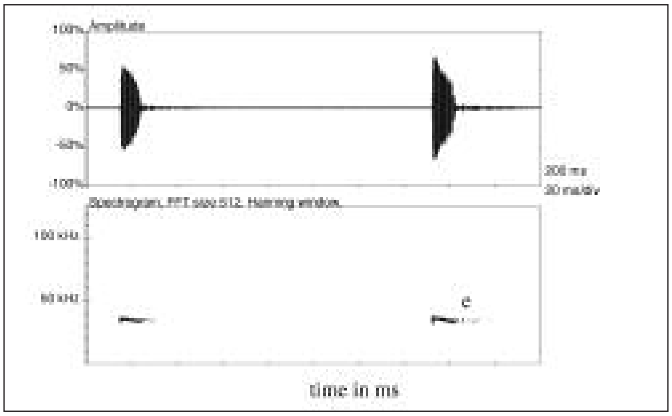

Figure 2: A time-amplitude and time-frequency display of two of the *Molossus molossus* calls shown in Fig. 1. The second call is the one with the arrow in Fig. 1. The smear immediately after the display of frequency change over time (e) is an echo of the call.

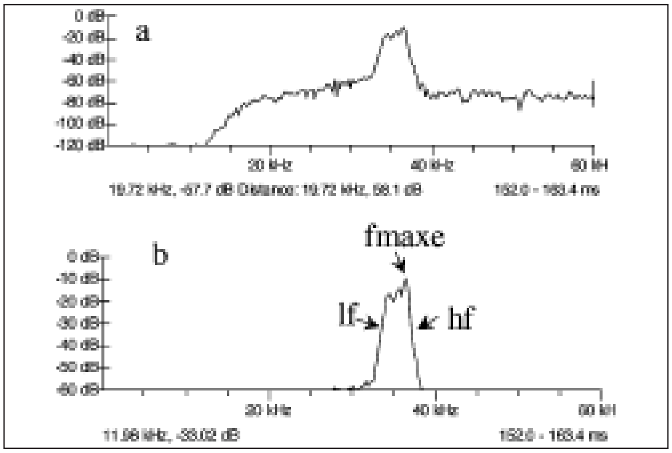

Figure 3: Two fast Fourier transform power spectra of the call marked with an arrow in Fig. 1, comparing a threshold of **a:** -120 dB, and **b:** -60 dB. In either case 0 dB is shown on the vertical axes. The -60 dB threshold eliminates background noise. Points of measurement of frequency with maximum energy (fmaxe), lowest frequency (lF), and highest frequency (hF) are shown.

In some settings, it will be possible to distinguish between general activity (Figs. 1) and feeding buzzes (Fig. 6), the high pulse-repetition rates associated with attacks on prey (Schnitzler and Kalko 2001). Bats typically lower the frequency of their echolocation calls during feeding buzzes (Fig. 6; Schnitzler and Kalko 2001), so the detector tuned to detect general activity may miss feeding buzzes. Some species change frequencies between search phase and buzz more than others. By choosing a broadband bat detector to monitor activity (or using the broadband output from a tunable detector), researchers will sacrifice some range because tuned detectors always have a greater effective range than broadband ones. On balance, however, a broadband detector should be more effective at providing data about feeding buzzes and general bat activity, remembering that supplementing acoustic with direct observations always pays off.

When using a broadband bat detector that provides a modified output such as frequency division, be sure to select the appropriate division ratio. Most frequency division bat detectors allow the user to select from a range of division ratios. For example, in using a divide-by-ten output to monitor bats using the 30-60 kHz bandwidth, the output will be in the 3-6 kHz range, readily audible to humans. But if the bats were using the 120-150 kHz range, the output (12-15 kHz) would be only faintly audible to humans, so a divide-by-twenty setting would be a better choice. A similar process can be used to choose between 4, 8, 16, and 32 times countdown on other detectors. Comparable decisions, for example about sampling frequency, must be made about the choice of settings used in other recording modes.

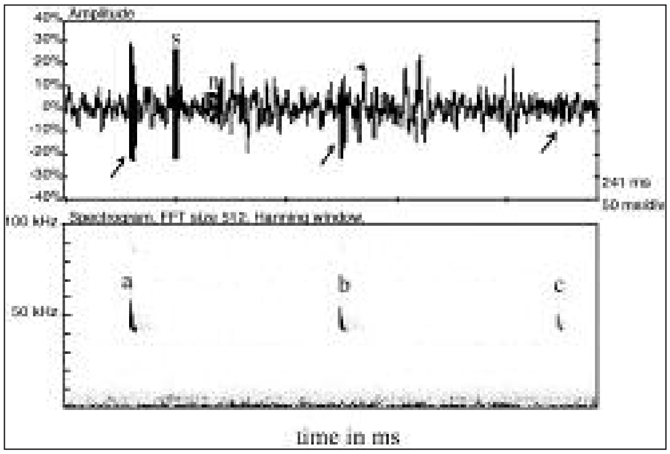

Figure 4: Three calls (arrows) recorded from *Pipistrellus kuhlii* showing variation in call strengths and signal-to-noise ratios. The heavy dark line reflects signal strength, and the dark dashed line reflects noise level. The first signal has the "best" signal-to-noise ratio, the third has the worst.

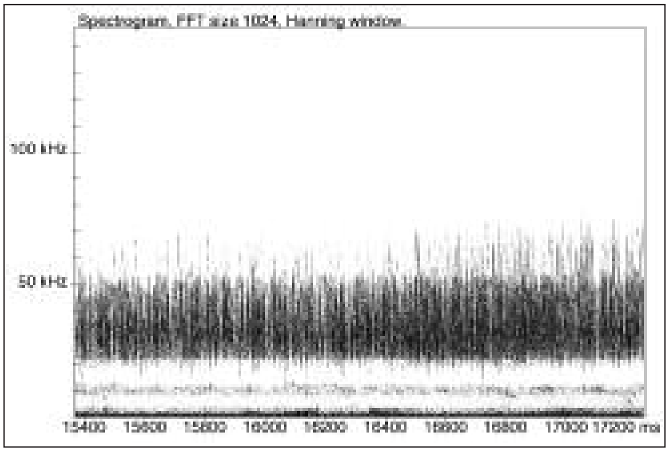

Figure 5: Calls recorded as tens of *Tadarida brasiliensis* emerge from a bridge roost in Reno, Nevada. In this situation it is impossible to distinguish between individual flights past the microphone.

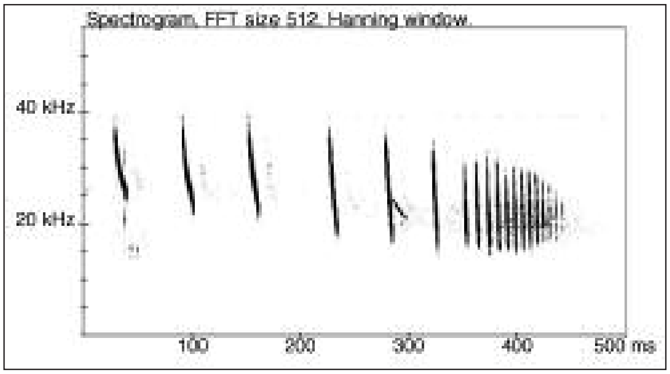

Figure 6: A feeding buzz recorded from a *Chaerephon pumilus* near Durban, South Africa.

The steps I follow when trying to determine the composition of the bat community in a study area are as follows: 1) from the literature, prepare a list of, and a key to, the bat species that might occur there; 2) from the literature, assess what is known about the echolocation calls (durations, frequencies, interpulse intervals) and behavior (high-intensity versus low-intensity calls) of these species; 3) at sites within the study area, use bat detecting systems to obtain an indication of how many call types are encountered; and 4) use recordings of the echolocation calls of captured, light-tagged (Hovorka et al. 1996), and released individuals to associate calls and species. In my opinion, recordings of bats held in the hand or released in a room are of minimal value in trying to assess the natural calls of bats.

In studies of bat activity, the data are usually reported as sequences of calls, passes, or feeding buzzes. The time between calls produced by an individual flying through the airspace (Figs. 1 and 4) should be used to establish criteria for grouping them into passes. For example, one could say that silent periods (no calls detected) of more than 4 times the average interpulse interval represent the break between one pass and the next. When more than one species is involved, differences in call features (frequency, duration, intercall interval) will inform criteria used to determine bat passes and different criteria may apply to different species. Researchers should specify the minimum numbers of calls used to identify a pass.

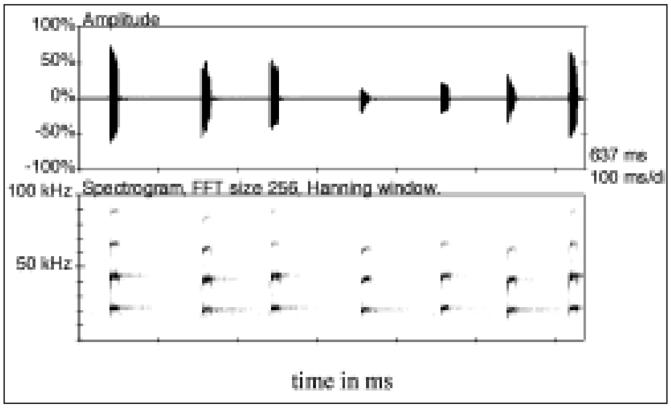

Figure 7: A sequence of 7 calls recorded as one *Saccopteryx bilineata* flew in the airspace sampled by the microphone. Note apparent differences in frequency between adjacent calls.

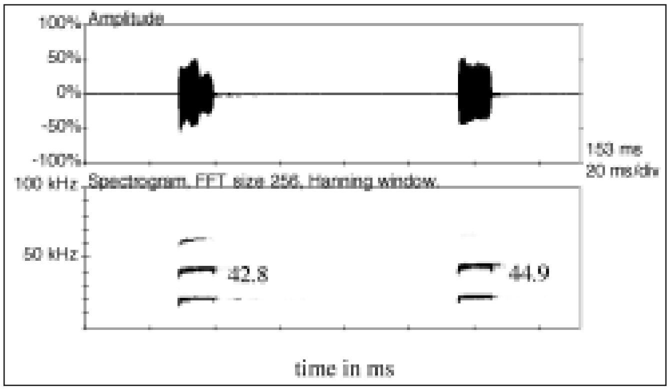

Figure 8: Expanded views of two of the *Saccopteryx bilineata* calls from Fig. 7. The time-amplitude displays demonstrate that the calls are not saturated (see below). Frequencies with most energy (fmaxe) in the calls differ between adjacent calls as indicated, demonstrating alternation of frequencies dominating echolocation calls.

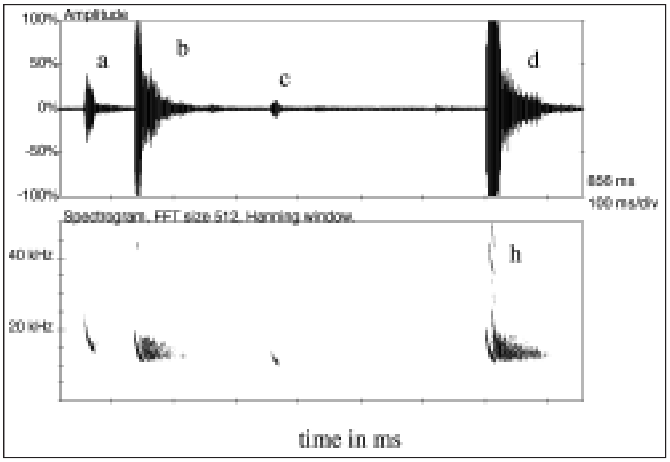

Figure 9: Three distinct calls (a, b, c) and one almost-simultaneous pair of calls (d) recorded from *Tadarida teniotis*. Time-amplitude displays reveal that calls b and d are much stronger than a or c, and differences in the level of recording influence the time-frequency display. Echoes are conspicuous as smears (see Fig. 2) after calls b and d.

INDIVIDUAL VARIATION

Although individual variation in echolocation calls complicates any research that depends upon monitoring echolocation calls to study the behavior of bats, the topic has received relatively little attention. Notable exceptions are the work of Rydell (1990), Obrist (1995) and Betts (1998) or that of Limpens and Kapteyn (1991),

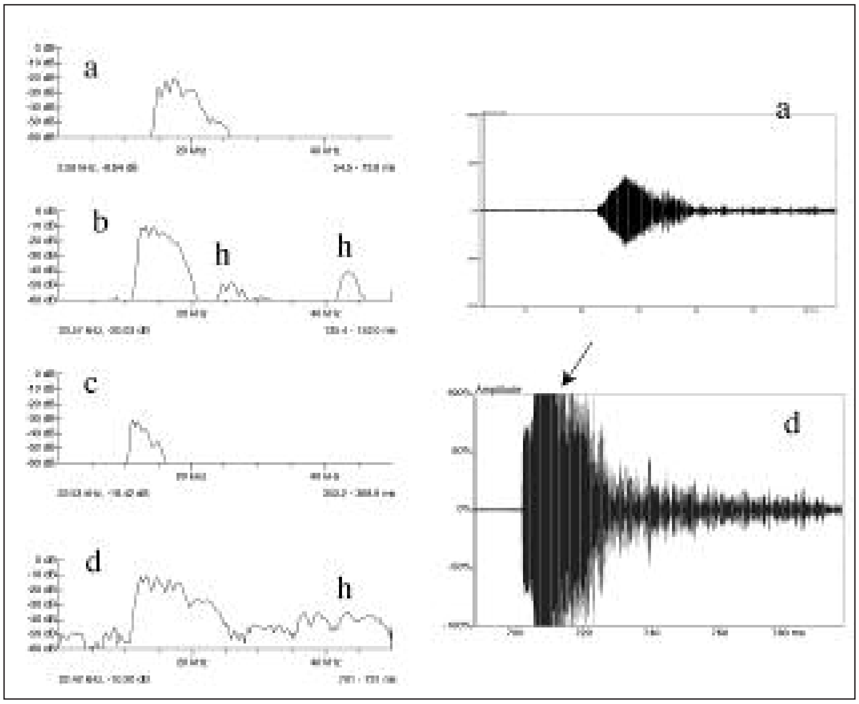

Figure 10: Fast Fourier transform power spectra for the calls shown in Fig. 9 (a, b, c, d, respectively) reveal the artificial harmonics (h) generated by "clipping" or saturation (arrow), shown in more detail in expanded time-amplitude displays of calls a and d.

Kapteyn (1993), Limpens and Roschen (1995), Zbinden (1989), and Limpens (2001). Changes in frequencies dominating echolocation calls during feeding buzzes (Fig. 6) influence the detectability of bats by narrow-band detectors. But in many species, individuals change frequencies between calls (e.g., Habersetzer 1981; Barclay 1983; Kalko 1995; Obrist 1995; Fenton et al. 1998).

Bats producing high-intensity echolocation calls often use harmonics or overtones to broaden the bandwidth of any call (Simmons and Stein 1980). In Fig. 7, there are 7 echolocation pulses with most energy between 40 and 50 kHz, but many are flanked above and below by other apparent calls which may be harmonics. Closer examination suggests that calls in the 40-50 kHz range alternate in frequencies (Fig. 8). To ensure that apparent harmonics are not artifacts of sampling (see below), one can examine an expanded time-amplitude display and associated patterns of frequency change over time (Fig. 8). Using this process, it is clear that the recordings are not saturated (see below) and that alternate calls differ in frequency (Fig. 8). Observation suggested that a single bat was responsible for the calls recorded here (Fig. 8).

In studies designed to assess variation in echolocation calls, larger samples of data from calls of different individuals and species will be required. Such details will be essential for exploring cases of cryptic species, distinguishable by their echolocation calls. The example of *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* (Barratt et al. 1997) demonstrates the identification of species by their calls.

IDENTIFICATION OF SPECIES BY THEIR CALLS

The focus of analysis is now the features of individual calls. Being able to examine the time-amplitude display as well as the associated spectrogram allows an observer to assess the impact of signal strength (time-amplitude) on spectrogram (Fig. 9). In Fig. 9, there are 4 calls, two (b and d) that are stronger than the others (a and c). Some workers have speculated that variation in alternate calls from the same individual reflect head movements (Siemers et al. 2001). When the signals are compared in more detail (Fig. 10), it is evident from the power spectra that the weaker calls (Fig. 10a, c) lack harmonics (h) present in the stronger ones (Fig. 10b, d). A closer examination (Fig. 10a, d) reveals "clipping" (arrows), times when the signal saturated (overloaded) the system. Clipping flattens sine waves, turning them into square waves and generating harmonics (h) that are artifacts. Unlike the situation in Fig. 8, the harmonics in Fig. 10 are artifacts of recording. In some cases, changes in time-frequency displays make it obvious that the calls of two species have been recorded, while the time-amplitude display reveals the quality of the recordings (signal-to-noise ratio) and the incidence of clipping or saturation. Features of time, interpulse interval, and duration (measured in ms) can be measured from the time-amplitude displays, informed by the time-frequency displays. Measurement of interpulse intervals requires a time-amplitude display of at least two pulses, while duration of individual calls can most accurately be measured from the expanded display of one pulse. Frequency features (lowest frequency, highest frequency, and frequency with most energy), including the presence of harmonics, are most accurately measured from power spectra (Fig. 3). In assessing highest and lowest frequencies one must report the dB level at which the frequency reading was obtained. While Fenton et al. (2002) used -55 dB from 0 dB to determine the highest and lowest frequencies in a call, Kalko and Schnitzler (1993) took a different approach. They used -6 dB and -15 dB (from the peak) readings to establish the highest and lowest frequencies in a call. The -6 dB marks half of the energy in the call. Others (e.g., Surlykke and Moss 2000) have followed this model. In a sample of 10 calls (of *Otomops martiensseni*), the difference in highest and lowest frequencies using these two approaches was < 0.5 kHz.

The challenge presented when trying to identify species by their calls is directly proportional to the number of species involved and the similarity between their calls. The situation is almost exactly as it is for identifying bats in the hand – some distinctions are easy, while others are more subjective. When published information provides an indication of the range of variation, researchers can assess the feasibility of making distinctions between species on the basis of call features. Often bat detectors are used in this way to assess the presence

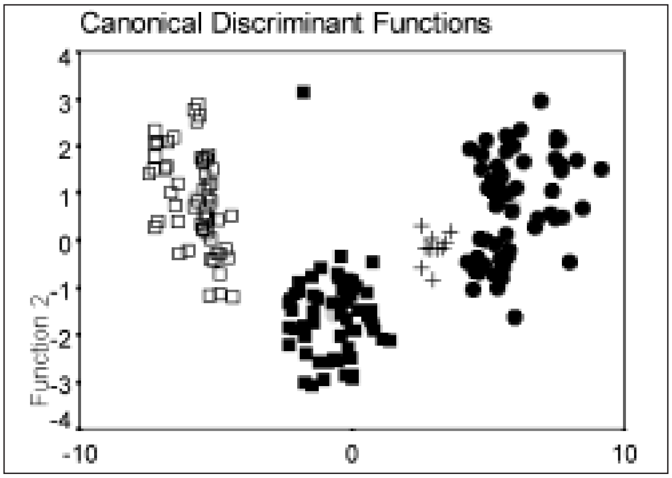

Figure 11: A discriminant function analysis indicates four distinct groups of frequency-modulated sweep calls representing at least four species. The data from each call are shown by species (□ – *Otomops martiensseni*, ● – *Chaerephon pumilus*, ■ – *Chaerephon* "other" [an as-yet-unidentified species of molossid], and + – *Tadarida aegyptiaca*). Function 1 accounted for 94.8% of the variance and was most strongly affected by lowest frequency in the calls.

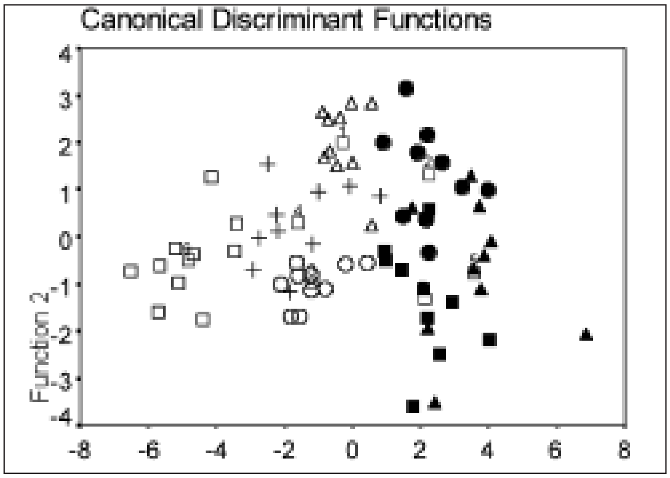

Figure 12: Discriminant function analyses of calls recorded from individual *Otomops martiensseni* emerging from one building roost in Durban, South Africa, (10 calls per sequence; calls from each individual identified by symbols) provides a clear separation of individuals by their calls. A multiple analysis of variance indicated significant interindividual variation in call features; and together, lowest frequency and frequency with maximum energy in the calls accounted for most of the variation.

of a species. In any event, local calibration of both equipment and bats is necessary to validate the operation.

When multiple analyses of variance (MANOVA) reveal significant within-species variation in call duration, frequency with most energy, lowest frequency, and highest frequency, discriminant function analysis can be used to assess the distinctiveness of calls from different taxa. This combination of approaches has been used by several workers (e.g., Obrist 1995; Masters et al. 1995; Pearl and Fenton 1996; Boughman and Wilkinson 1998), while others have used multinomial logistic regression (e.g., Press and Wilson 1978) or artificial neural networks (e.g., Jones et al. 2000). To obtain these data, the researcher must use a broadband bat detecting system working with a recording device (whether analogue or digital, tape or card) in combination with apparatus (software or hardware) that will allow extraction of this information. Today, most researchers probably will use a computer and software to analyze the calls, recording the data on a spreadsheet for appropriate statistical analysis. Different programs will provide different levels of accuracy for the data from any call and the similarity between species will set the standard required for any study.

The situation is exemplified by plotting the results of a discriminant function analysis of 10 to 60 calls recorded from each four species of molossids from South Africa (Fig. 11). In this case, the analysis accurately assigned all calls to species. A similar situation, significant individual variation followed by discriminant function analysis, demonstrates accurate assignment of calls to individuals in most cases (Fig. 12).

STUDIES OF ECHOLOCATION BEHAVIOR

When the purpose of a study is to describe and document echolocation behavior, detailed information about calls (as above) is required and should be tied to data about the behavior of bats. Simultaneous use of stroboscopic photography and recording has been used successfully in this context (e.g., Kalko and Schnitzler 1993; Kalko 1995a, b; Schnitzler et al. 1994; Britton and Jones 1999).

Before embarking on a study using the echolocation calls of bats, whether as the means to an end or as the end itself, consider the following steps:

1) Clearly articulate the question(s) you want to answer. It may be helpful to identify a guiding hypothesis and the testable predictions it generates. Assess whether or not the data you will obtain will allow you to test the predictions of your hypothesis.

2) Ensure that the bats in question are known to produce echolocation calls that are readily detectable by the system you are considering.

3) If you choose to use a narrowband (tunable) bat-detecting system, recognize that you may not be able to reliably distinguish bat passes from feeding buzzes. Furthermore, at any given frequency, you may be limited in your capacity for distinguishing between species using similar calls.

4) If you select a broadband system, ensure that your choice of system (from hardware to software) will give you the resolution you require. Present information about the settings you used during recording and analysis.

5) Clearly articulate the procedures you followed to collect data and the criteria you used in their analysis to ensure that others can replicate your study. You may want to pay special attention to the recognition of artifacts such as clipping and aliasing.

6) Whenever possible, watch the bats whose echolocation calls you are monitoring.

Be sure that your reading includes both classics (e.g., Griffin 1958; Sales and Pye 1974) and appropriate most recent papers. There is no reason for anyone to repeat the mistakes of others.

ACKNOWLEDGEMENTS

I thank the organizers of the symposium for including me on the program as well as those who read and commented on earlier version of this manuscript, including Rafa Avila-Flores, Enrico Bernard, Stefania Biscardi, Brian Keeley, Herman Limpens, Liz Reddy, and Hannah ter Hofstede. My research on bats has been supported by research grants from the Natural Sciences and Engineering Research Council of Canada.

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EUROPEAN AGENCY PERSPECTIVES: EXPECTATIONS FROM BAT DETECTOR STUDIES

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European states approved the necessity of protecting bats through legal regulations, including the European Union Habitats Directive and various national laws and international agreements, such as the Berne Convention, Bonn Convention and EUROBATS Agreement. To fulfill international obligations and identify appropriate measures for bat conservation, more autecological research and a bat monitoring program is needed all over Europe. Bat detectors are useful tools for collecting data on the abundance of bat species, their behavior, and habitat requirements. In Europe, most fieldwork is done by amateur bat workers. Compared to professional researchers, amateurs have less time and little money, especially in eastern European countries, which are in economic transition. Therefore bat detectors should not only have a high technical standard, but also an easy-to-use design and a reasonable price. Besides technical developments in detectors, bat conservation requires an admiration of the animals and application of available techniques. For this reason, more appropriate information, education and especially bat detector training is needed for bat workers all over Europe.

Key words: amateur bat workers, bat conservation, bat detectors, international obligations, monitoring, research

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INTRODUCTION

The European bat fauna consists of about 34 species of 3 microchiropteran families: Rhinolophidae (5 species), Vespertilionidae (27-29 species), and Molossidae (1 species – Mitchell-Jones et al. 1999). Compared to regions in the tropics or subtropics, the diversity of European bats is low and includes only a few endemic species. Nevertheless, this group of mammals has been the subject of scientific research and conservation efforts for more than 100 years. During the last decade, bats have become very popular, at least in central Europe, and today they are among the most important organisms used in environmental impact assessments, public awareness about natural history, and international cooperation for nature conservation. In all European countries, bats are protected by law.

New emphasis on bats in the context of nature conservation requires a need for more information about their behavior and ecology. Data are needed for local, regional, national, and international purposes. However, the availability of data is very different across European regions and covers different time periods and species. More fieldwork is needed.

Bat detectors are useful tools for investigating most species in nearly all European habitats. In this paper, I discuss the role of bat detector surveys to collect data for nature conservation in Europe and describe the ideal technical profile of future instruments and support.

LEGAL OBLIGATIONS FOR BAT CONSERVATION IN EUROPE

In Europe, international agreements for the conservation of wild animals have a 100-year history (Lutz et al. 2000). Since the 1970s, when the Ramsar Convention was negotiated and the U.N. Conference in Stockholm took place, several initiatives have led to the development of a network of international regulations in Europe. In 1979, the Berne Convention on the conservation of European animals, plants, and habitats, and the Bonn Convention on migratory species were negotiated, both listing bat species in their annexes and urging legal protection for them. That same year, the European Community (now called the European Union) implemented the Bird Directive, which is a legally binding framework for bird conservation in EU member states. As many EU countries became members of the Berne Convention, they adopted the key elements of the Convention as legally binding through the EU Habitats Directive in 1992. The Habitats Directive is complementary to the Bird Directive, and both demand special protected areas, which together form a European network called "Natura 2000."

Name	Character	Targets	Members
Convention on Biological Diversity (CBD)	International agreement on principles and measures that should be followed or implemented	Conservation and sustainable use of ecosystems, species, and genetic diversity; equitable sharing of benefits from genetic resources	183 parties from all over the world
Convention on the Conservation of European Wildlife and Natural Habitats (Berne Convention)	International agreement on principles and measures that should be followed or implemented	Conservation of European animals, plants, and habitats	79 parties from Europe and Africa
Directive on the Conservation of Natural Habitats and of Wild Plants and Animals (EU Habitats Directive, 92/43/EWG)	Legally binding framework which must be implemented under the national law of EU member states	Conservation of European animals, plants, and habitats by a network of protected areas and regulations for taking or use	All 15 EU member states. 12 countries are candidates for future EU membership
Convention on the Conservation of Migratory Species of Wild Animals (CMS, Bonn Convention)	International agreement on principles and measures that should be followed or implemented	Conservation of migratory species, especially those which are endangered	45 parties from all over the world (except North America)
Agreement on the Conservation of Bats in Europe (EUROBATS)	International agreement on principles and measures that should be followed or implemented	Conservation of all bats on the continent of Europe	26 countries from Europe and the Caucasus region

Table 1: International legal regulations for bat conservation in Europe.

The intent of the Bonn Convention is to lead to agreements on the species listed in its Annex 2, leading to improved conservation status of these animals in each specified region. Since all European bats are listed in the annex, it was convenient to form a regional agreement on them. The Agreement on the Conservation of Bats in Europe (EUROBATS) was negotiated in 1991 and came into force in 1994. Every third year, there is a Meeting of Parties to assess national activities and financial matters and to adopt a conservation and management plan for the next three years. For example, the Meeting of Parties passed recommendations for uniform methods for Europe-wide bat population surveys and formulated research programs for species (e.g., *Myotis dasycneme, Pipistrellus nathusii*), as well as for habitats (e.g., underground habitats, forests). All member states are obliged to monitor bat populations and promote autecological research (Boye et al. 1999). Today, the EUROBATS Agreement is managed as an independent organization under the auspicies of the United Nations Environmental Program (UNEP). Albania, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Georgia, Germany, Hungary, Ireland, Lithuania, Luxembourg, FYR Macedonia, Malta, Moldova, Monaco, The Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Sweden, Ukraine, and the United Kingdom participate as Parties to the Agreement.

The 15 EU member states have more obligations to be fulfilled in bat conservation. The Habitats Directive must be implemented through corresponding national laws, and it demands full protection for all bat species and special conservation measures for some of them. Species in Annex 2 of the Directive (all European *Rhinolophus* species, *Barbastella barbastellus*, *Miniopterus schreibersii*, *Myotis bechsteinii*, *M. blythi*, *M. capaccinii*, *M. dasycneme*, *M. emarginatus*, and *M. myotis*) must be conserved by designating special protected areas for important populations. Populations in specially protected areas as well as national populations of all bat species, must be monitored using surveys at least every sixth year. In the case of projects with a possible influence on the protected areas, even if they are not within the protected area itself, a detailed impact assessment is required in advance. Relevant gaps in knowledge about the ecology and conservation of bats must be the focus of research (Petersen 2000). Implementation of the Habitats Directive is currently in progress. National governments and EU authorities are debating the requirements of the protected areas network "Natura 2000" and monitoring programs. When the 12 candidate states become new members of the EU, the Habitats Directive will become legally binding on them, as well.

The EU has further regulations that force member states to implement nature conservation measures. For example, landscape planning and the assessment of environmental impact of a project must follow specific standards. In countries like Germany, these standards are very elaborate and in many cases involve bats as indicator species (Plachter et al. 2002).

The obligation to adhere to principles of international agreements, especially the legally binding EU Habitats Directive (Table 1) is taken as part of the implementation of the Convention on Biological Diversity in Europe. There is no other program to ensure the conservation of bat diversity under this umbrella convention. However, research should be conducted to evaluate bats as indicators of ecological sustainability in resource management (Paoletti 1999). For example, if adequate data and assessment techniques were available, bats could be used as indicators for sustainable forestry management under the

Montreal Process (Taylor and Doran 2001).

To summarize, there are binding international obligations on nearly all European countries to promote bat conservation. This includes the need to collect data to monitor populations and their habitats and to improve our knowledge about the species' ecology. Members of all the international provisions named in Table 1 are Denmark, Finland, France, Germany, Luxembourg, The Netherlands, Portugal, Sweden, and the United Kingdom. The most important agreements are the EU Habitats Directive and the EUROBATS Agreement, which both provide incentives for increasing research and monitoring activities (Table 2).

Action	Item	Background
Autecological research	behavior and biology	Berne Convention, Bonn Convention, EUROBATS
	relations between abundance and environmental factors	Berne Convention, EUROBATS
	key factors for conservation	Berne Convention, EU Habitats Directive, Bonn Convention, EUROBATS
Site protection	identification of key sites	Berne Convention, EU Habitats Directive, EUROBATS
Monitoring	species population sizes, trends, and distributions	CBD, EU Habitats Directive, EUROBATS
	population sizes and trends in special protected areas	EU Habitats Directive
	presence of species in certain habitats	EU Habitats Directive, EUROBATS
	impact of conservation action	EU Habitats Directive
Landscape planning and impact assessments	presence and abundance of species in certain habitats	EU Habitats Directive (only in protected areas), EUROBATS

Table 2: International obligations for fieldwork on bats in Europe.

METHODS AND PROTAGONISTS OF FIELDWORK ON BATS

Research, monitoring, or impact assessment work on bats typically involves a number of field methods. Each method is to some degree biased, and no single technique will lead to a complete and unbiased picture of the bat population of an area (Mitchell-Jones and McLeish 1999). In addition, currently available methods are different in terms of cost and the training needed by the users (Limpens and Roschen 1996, 2002). The methods are:

- roost surveys (buildings, trees, bat boxes, and underground habitats)
- bat detector surveys conducted to find roosts, foraging areas, flight paths, and mating sites
- capturing bats in foraging habitats using mist nets and harp traps
- direct observation with night-vision devices
- individual marking such as ringing/banding and passive, integrated transponder (PIT) tagging
- telemetry
- activity monitoring using automated stationary bat detectors or infrared light curtains.

For all of these methods, environmental factors should be recorded to assess the likely causes of observed behavioral or population trends.

Among these techniques, the use of bat detectors is most appropriate in many situations for three reasons:

1) Detectors provide opportunities for data collection at different levels of quality. Even a relatively untrained person is able to find places where bats concentrate. This is a starting point for further investigation. Many users of detectors can identify at least some European bat species. Based on bats' vocalizations, fully trained workers can assess activity, microhabitat, and hunting efficiency and can distinguish between most species, especially when using time-expansion detectors. However, identification of the sibling pairs *Plecotus auritus/P. austriacus*, *Myotis myotis/M. blythii*, and *Myotis mystacinus/M. brandii* to species based on sound and flight behavior is very difficult.

2) Bat detectors (except professional equipment) are easy to carry and can be used in many situations.

3) Nature conservation law does not limit bat-detector surveys. Most other techniques require official permission or permits in European countries.

In EU countries, bat detectors will likely be an important tool used to fulfill the obligation of reporting implementation of the Habitats Directive. Results of detector surveys will be used to assess the conservation status of bat species in a country and for surveying local bat populations in protected areas (Dense and Mayer 2002).

A review of possible methods for research and survey work appropriate for the use in the field and relevant to bat conservation highlights the importance of bat detectors and amateur bat workers for autecological research and monitoring. Indeed, many volunteers contribute to bat research and conservation programs in Europe. Many bat workers use bat detectors to survey foraging areas or as an additional tool for roost surveys and during mist netting. Most workers use heterodyne detectors due to their releatively low cost and because of the availability of reference calls on CD. Amateurs typically have less time and money to spend on equipment than professionals at universities or consulting companies. However, many bat workers (both professionals and amateurs) seek to improve their skills using detectors and would prefer time-expansion instruments.

It is unlikely that European nature conservation agencies will ever be in the position to employ professional staff to collect all of the required data on bats. Therefore, volunteers who collect reliable data and provide them for the fulfillment of international obligations are needed. However, conditions for volunteer bat work vary between different European countries. EUROBATS member states can be divided into four groups depending on the number of bats and the economic situation of the countries (Table 3). In Western Europe, where workers typically have cars and bat detectors, the number of amateur bat workers is influenced by the bat fauna: Few species and low abundance in northern countries do not fascinate as many people as in more southern locations, where there are more species and greater abundances. In southern Europe, where bat numbers are highest, the number of bat workers depends very much on the economic situation. Where income and mobility are moderate, academics in universities, professional conservation biologists, and amateurs all contribute to bat studies. However, in those countries in economic transition, volunteer bat work is virtually absent because of low incomes, poor mobility, and time constraints. For these reasons, the development of international bat monitoring programs must take into account the economic situation of the people who are expected to participate. Detectors must have a threshold technical standard in sensitivity and handling to ensure the collection of reliable data, but they should also have reasonable prices so as to be accessible for as many people as possible.

CONCLUSIONS

European agencies consider bat detectors to be valuable tools for collecting field data on bats relevant to conservation issues. The development of detectors over the last two decades has contributed to our current knowledge about bats and has attracted more voluteers to conduct fieldwork. Training in the use of detectors improves observers' abilities to identify bat species and find roosts by following flight paths. Monitoring schemes should be developed which lead to comparable results across Europe. The distribution and application of bat detectors should be promoted further. Besides technical and financial aspects, other considerations include more education and training, and reference guides for bat workers to compare their own observations and records with others. European agencies for nature conservation should endorse the development of such reference guides because reliable data, data compilation, and elaborate analyses are in their own self-interest. The Internet is an appropriate platform accessible from everywhere in Europe. A second requirement is more support for bat detector training workshops. From 1999 to 2001, the German government funded workshops in 10 eastern European countries in economic transition as a special contribution to the implementation of the EUROBATS Agreement. These were a great success and proved the value of bat detectors for international cooperation in bat conservation in Europe.

	1st group	**2nd group**	**3rd group**	**4th group**
examples	Germany, The Netherlands, UK	Sweden[1], Finland	Portugal, Hungary, Croatia	Romania, Ukraine, Georgia
bat species	< 20	< 15	> 20	> 20
bat abundance	intermediate	low	high	high
availability of bat detectors	good or very good	good	intermediate or good	very bad
individual mobility	high	high	high or moderate	low or very low
average income	high	high	moderate	low
number of amateur bat workers	several hundred	few	few	very few

[1] The southernmost part of Sweden has conditions of the 1st group.

Table 3: Conditions for bat fieldwork in EUROBATS member states.

ACKNOWLEDGMENTS

I thank Bat Conservation International for the invitation to present European agency perspectives at this workshop. Mark Brigham, Gareth Jones, and another reviewer provided comments that improved the manuscript.

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GEOGRAPHIC VARIATION IN THE ECHOLOCATION CALLS OF BATS: A COMPLICATION FOR IDENTIFYING SPECIES BY THEIR CALLS

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Variation in the echolocation calls of bats in different populations of the same species (i.e. geographic variation) may complicate attempts to use echolocation to identify free-flying bats to species. Such variation occurs in several species. Variation in echolocation calls is correlated with variation in body size and is also associated with differences in habitat type on a local level. Factors such as environmental conditions (e.g., relative humidity), prey types (e.g., size and defense mechanisms), foraging behavior (e.g., gleaning versus aerial hawking), and the presence of other bats may also translate into variation at larger geographic scales. We suggest ways to account for potential call variation when using a reference library to identify bats to species.

Key words: Body size, Chiroptera, foraging, habitat, prey, survey techniques

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INTRODUCTION

The echolocation calls of free-flying microchiropteran bats have proven to be extremely useful for studying the ecology and behavior of these otherwise elusive animals. For decades, systems that detect echolocation calls have been used for various purposes, some of which require assigning calls to particular species or species groups. Bat detectors have been used to address both basic and applied issues, including questions about the relative abundance of different species in an area, the diversity of species and how diversity varies geographically, the ecological or morphological structure of bat communities, the presence of rare or endangered species, the use or selection of particular foraging habitats by different species, and the determination of critical habitats. There is also the potential for identifying cryptic species by their echolocation calls (e.g., Jones

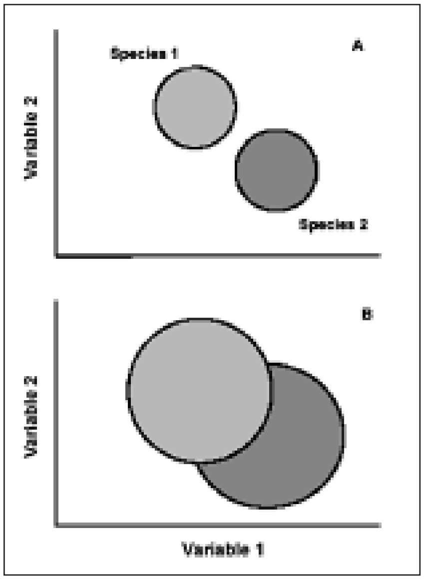

Figure 1: Variation in two characteristics of echolocation calls within and between two species of bats. **A:** Variation within each species, depicted as the shaded circles, is relatively small and calls can be unambiguously assigned to one species or the other. **B:** The mean values for the two call variables are the same as in A, for each species, but there is greater within-species variation. This means that there is overlap among the calls of the two species. Calls with characteristics in the zone of overlap cannot be assigned to a particular species with 100% certainty.

and van Parijs 1993).

Early studies using bat detectors indicated that different species of bats use different echolocation calls, thus suggesting that free-flying individuals could be identified on the basis of their calls (Ahlén 1981; Fenton and Bell 1981). However, one problem in using echolocation calls to identify species of bats is that the characteristics of the calls of each species (e.g., duration, bandwidth, frequency, harmonic structure) vary. To address questions requiring species identification, researchers rely on the fact that variation in call structure is generally greater among species than within species. Within many bat communities, the calls of some species are distinctive (Fig. 1A), and even though there is variation within each species, calls can be assigned unambiguously to particular species. In most cases, however, the call characteristics of various species overlap because on average the calls of different species are very similar to one another, or because there is greater variation in the calls of each species (Fig. 1B). Assigning calls to a particular species is less certain under these circumstances. Calls that fall within the zone of overlap (Fig. 1B) may be assigned to a species with a probability less than one, with the probability increasing the closer the combination of traits is to the mean value for that species. By definition, there is error involved in making such classifications. Often, multivariate statistical techniques such as discriminant function analysis (DFA) or neural networks are used to determine the probability of assignment (e.g., Jones et al. 2000; Parsons and Jones 2000; Vaughan et al. 1997).

Variation in the echolocation-call characteristics of a particular species of bat clearly increases the uncertainty of identifying calls of unknown, free-flying individuals to that species. Such variation occurs for a number of reasons, including variation within a population related to characteristics of the individual, such as age, gender, and body mass (Betts 1998; Brigham et al. 1989; Jones et al. 1992; Jones and Ransome 1993; Obrist 1995). Some of these factors may cause variation in calls from different locations (e.g., body mass), while others will not (e.g., age and gender), unless population structure varies (e.g., Barclay 1991). The purpose of this review is to consider geographic variation, especially among populations within a species, and to make recommendations as to how to account for it when conducting studies that depend on identifying bats by their echolocation calls.

By geographic variation we mean variation in the structure of echolocation calls of individuals of the same species recorded in different locations. It is a potential problem if call characteristics from one population are used to identify the calls of unknown individuals from another population (Law et al. 2002). Most studies rely on a set of calls from known individuals (a reference library) to establish the characteristics used to identify unknown bats recorded by the monitoring system.

Suppose there are two species in our study area and we base species identification on the call characteristics determined from another location (Fig. 2). Suppose further that the characteristics of the calls of the two species are distinct, but call characteristics from individuals in our study populations differ from those in the reference library. Such geographic variation could result in the inability to identify some calls and misidentification of others, depending on the amount of geographic variation (Fig. 2).

While geographic variation is most commonly thought of on a large scale, an analogy being the dialects of humans and birds, there is relatively little information regarding this for the echolocation calls of bats. A large part of the problem is a lack of consistency in recording techniques and equipment among studies. There are, however, considerable data regarding smaller-scale geographic variation and variation caused by other factors that may result in large-scale geographic differences. We thus examine these sorts of variation, make predictions

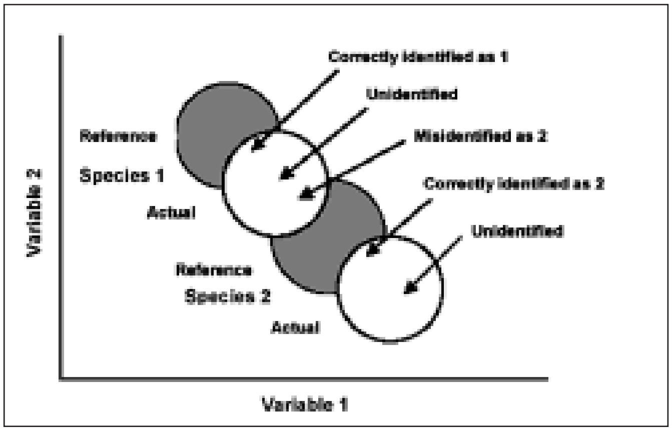

Figure 2: Potential problems associated with geographic variation in the characteristics of the echolocation calls of two species of bats. Characteristics from the reference library for each species (shaded circles) are distinctive, but differ from the characteristics of the calls from the study populations (open circles). Because of this, some calls will be unidentifiable (they do not fall within either reference set), and others will be misidentified (they fall within the reference set of the other species).

about how they may translate to larger geographic scales, and examine the evidence that such large scale, interpopulation variation exists.

SMALL-SCALE VARIATION IN ECHOLOCATION CALLS

Insectivorous bats vary the design of their echolocation calls depending on the task they are performing and the type of information they require. Calls change in structure as a bat shifts from searching for prey to approaching and attacking it (e.g., Kalko 1995; Kalko and Schnitzler 1993). Bats also vary call design depending on the nature of the habitat (but see Pavey et al. 2001 for a situation where variation was not present). Calls from a particular species are often higher in frequency, broader in frequency sweep, and shorter in duration the more cluttered the habitat is, the smaller the gap in a forest is, or in general the closer the bat is to vegetation (e.g., Barclay et al. 1999; Kalko and Schnitzler 1993; Obrist 1995; Schnitzler and Kalko 2001; Siemers and Schnitzler 2000; Figs. 3 and 4). This has been attributed to the need for bats to avoid overlap between the outgoing echolocation pulse and the returning echo (Kalko and Schnitzler 1993), and therefore, the need to use shorter calls when bats are closer to targets. In addition, in more open habitats, longer, lower-frequency calls are more efficient for longer-range prey detection (Barclay and Brigham 1991).

The height at which a bat is flying also influences the design of its echolocation calls. For several species, individuals flying low nearer the ground produce shorter, higher-frequency calls, perhaps for similar reasons to the correlations noted above (Jensen and Miller 1999; Zbinden 1989; Fig. 5).

Thus, at a local level, bats foraging in different locations may differ in the echolocation calls they emit, thereby generating variation in call characteristics in a population. Such variation presumably occurs for the same reasons between more distant locations if the foraging habitats differ, or if individuals forage at different heights. This might occur between populations if the species occupies a range of habitat types (e.g., forests and grasslands), or if prey distributions differ in different locations and the optimal foraging habitat for bats thus varies.

OTHER FACTORS INFLUENCING ECHOLOCATION CALL VARIATION

Echolocation calls vary among species of insectivorous bats in relation to body size (e.g., Barclay and Brigham 1991; Heller and von Helversen 1989). This may be for purely physical reasons (large vocal chords can not produce high frequency sounds), or for adaptive reasons associated with prey size and detection (e.g., Barclay and Brigham 1991; Guillén et al. 2000). Within a species, a similar correlation between echolocation call characteristics and body size occurs (Barclay et al. 1999; Guillén et al. 2000; Fig. 4). In *Miniopterus schreibersii*, individuals with longer wings have lower frequency calls (Jacobs 1999). Body condition (e.g., body mass relative to forearm length) also appears to influence call characteristics in some species (Guillén et al. 2000; Jones et al. 1994), but not others (Russo et al. 2001). If body size or condition varies geographically, as occurs in some species (Parsons 1997; Patriquin 2001), then call design may also vary geographically, for example with latitude, although to our knowledge no explicit data exist regarding this.

Environmental factors also influence the echolocation calls of bats. For example, high relative humidity attenuates high-frequency sound more than low frequency sound (Griffin 1971), and there may thus be an advantage to producing lower-frequency calls in areas of high humidity. Indeed, such a correlation exists for at least one species (Guillén et al. 2000).

A bat's foraging style and type of prey may also influence the echolocation calls it uses. Some species are able to glean insects from vegetation or the ground, as well as capture insects in the air (e.g., *Myotis evotis*; Faure and Barclay 1994). In many gleaning species, prey detection is accomplished using sounds produced by the prey, and/or visual cues, in addition to echolocation. Gleaning also involves flying in closer proximity to clutter than

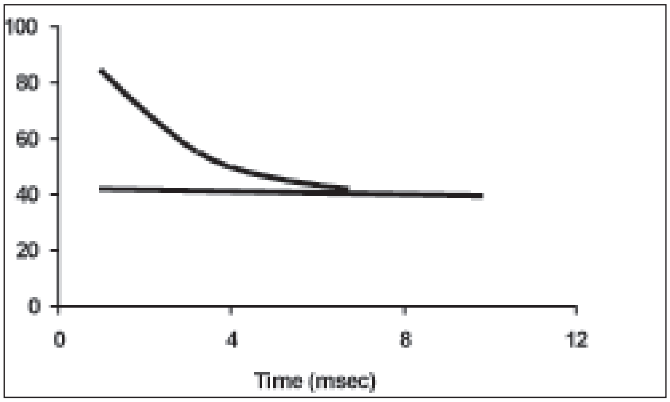

Figure 3: Variation in the echolocation calls of *Pipistrellus kuhli*. The lower call is typical of individuals flying in open habitats, while the upper call is typical of individuals flying close to vegetation (data from Kalko and Schnitzler 1993).

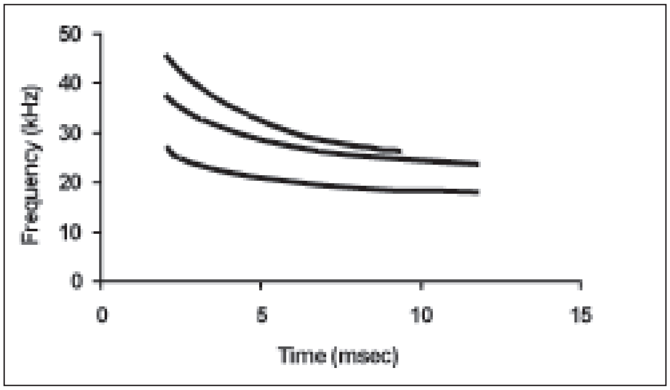

Figure 4: Variation in the echolocation calls of *Lasiurus cinereus*. The lower call is typical of individuals from Manitoba, Canada. The upper two calls are typical of individuals from the Hawaiian Islands. Hawaiian individuals are approximately half the mass of mainland individuals and belong to a different subspecies. The upper call is from an open site and the middle call is from a more cluttered site (data from Barclay et al. 1999).

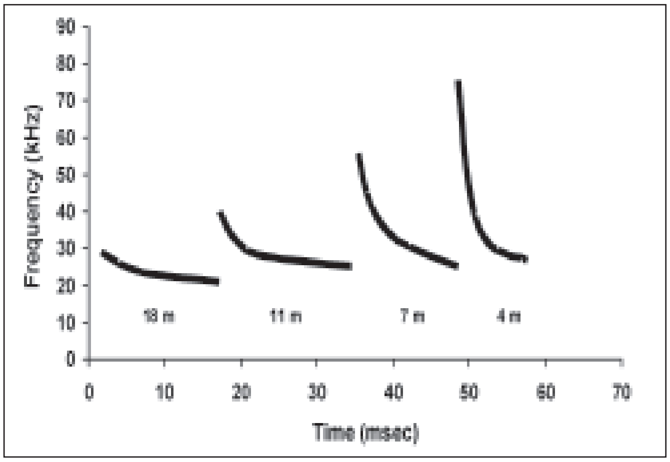

Figure 5: Variation in the echolocation calls of *Eptesicus serotinus*. From left to right, calls are from individuals foraging at progressively lower heights above the ground (height indicated under each call). Redrawn from Jensen and Miller 1999.

does aerial hawking. It is thus not surprising that species that use both foraging styles vary their calls depending on their foraging behavior (Faure and Barclay 1994). *Myotis evotis*, for example, uses quieter calls and does not produce a feeding buzz when gleaning. If conspecifics in different locations forage in different ways, perhaps because prey resources differ, for example, then the echolocation calls they use may also differ.

Prey characteristics may affect the type of echolocation calls used by bats (Leippert et al. 2002). Some prey, such as some moths, can detect the echolocation calls of bats, and this defense may have favored the evolution of specific echolocation features such as high or low frequency, low intensity, or short duration (Fenton and Fullard 1979). Characteristics of different prey communities within the geographic range of a bat species may thus have resulted in geographic variation in echolocation calls. Because the frequency of a sound and its corresponding wavelength also influence the strength of the returning echo from targets of different size (Hartley 1989), the size of prey available in an area could also potentially favor certain echolocation call designs. Specifically, the need to detect smaller prey should favor the use of higher-frequency calls. If so, and prey size varies over a bat species' range, echolocation calls might also vary geographically.

Finally, the presence of conspecifics and other species may influence the type of echolocation calls individuals use. Individuals of several species modify their calls in the presence of conspecifics (Obrist 1995). At a minimum, this will increase the amount of variation in call characteristics in a particular location and thus make species identification more difficult.

GEOGRAPHIC VARIATION AT A LARGER SCALE

Although few studies have specifically assessed geographic variation in bat echolocation, there is evidence that the echolocation call characteristics of some species of bats do vary geographically across the species' ranges. Among various rhinolophid and hipposiderid bats that use constant frequency (CF) echolocation calls, considerable geographical variation occurs in the CF frequency (e.g., Francis and Habersetzer 1998; Guillén et al. 2000; Heller and von Helversen 1989; Figs. 6 and 7). Variation in body size does not provide a satisfactory explanation in many of these cases. However, among the subspecies of hoary bat (*Lasiurus cinereus*), body size does correlate with significant geographic variation in echolocation call characteristics (Barclay et al. 1999; Fig. 4). There is also geographic variation in call structure in both species of New Zealand bats, and this variation may be related to body size or subspecies differences, although data are limited (Parsons 1997). In other cases, geographic variation in call structure has been documented in various species, but no functional explanation has been determined (Murray et al. 2001; Thomas et al. 1987).

CONSEQUENCES OF GEOGRAPHIC VARIATION AND RECOMMENDATIONS

Despite limited study, there is evidence that the echolocation calls of a number of species vary geographically and there are both physical and adaptive reasons to expect such variation. Although in some cases, geographic variation may not be great enough to cause

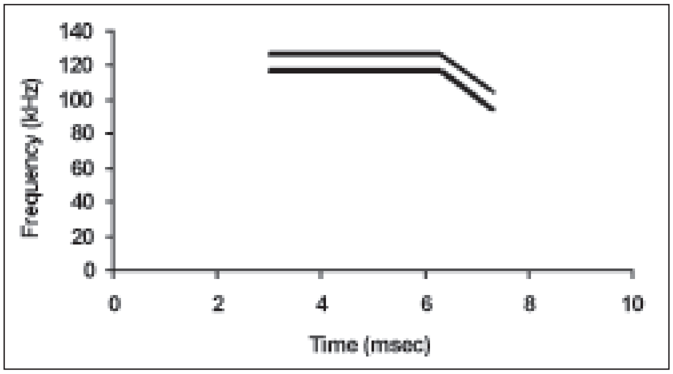

Figure 6: Geographic variation in the echolocation calls of *Hipposideros cervinus*. The upper call is typical of individuals from peninsula Malaysia, and the lower of individuals from Sabah, Borneo (data from Francis and Habersetzer 1998).

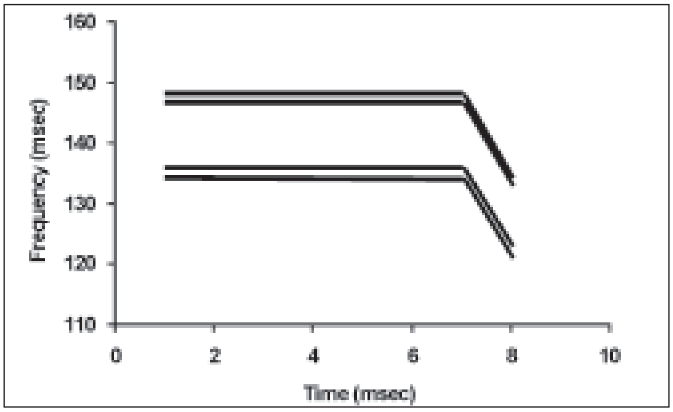

Figure 7: Variation in the echolocation calls of *Hipposideros ruber*. The upper two calls represent average values for individuals from a colony in Rio Muni, and the lower two calls are for individuals from a colony on the island of São Tomé. In each pair, the upper call is typical of males and the lower is typical of females.

problems in identification (Murray et al. 2001), we believe that studies using echolocation calls to identify individuals to species need to take geographic variation into consideration. If the reference calls used to identify unknown calls to species come from locations other than the study area, there is the potential for a mismatch between the reference calls and those actually used by the same species in the study area (Fig. 2). If so, at best the ability to accurately identify calls to species will be reduced. The number of unidentifiable calls may increase because they fall outside the set of values determined from the reference library. Assigning a probability that an unknown call belongs to a particular species may also be inaccurate. At worst, misidentification will occur (Fig. 2).

To maximize the accuracy of species identification, we recommend the following:

- The library of reference echolocation calls from known individuals should be drawn from individuals captured and recorded in the study area.
- When this is not possible, reference calls should come from locations as close to the study area as possible, and from areas that have the same foraging habitats as the study area.
- Reference calls should come from individuals from the subspecies that is present in the study area, and from individuals that are the same body size and condition.
- Reference calls should be recorded from free-flying bats of both genders in the variety of habitats to be studied. Ideally, reference calls should be matched to unknown calls from the same habitat.
- Reference calls should be taken from a significant number of individuals so as to incorporate the variation inherent in the population.
- The recording and analysis equipment and technique used for the reference calls should be the same as those used for the unknown calls.

ACKNOWLEDGEMENTS

We thank L. Hollis, T. Luszcz, D. Solick, J. Wilson and an anonymous reviewer who provided valuable comments on a previous draft of this paper. Our own research has been supported by grants from the Natural Sciences and Engineering Research Council of Canada, and the Universities of Calgary and Regina.

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BAT CALL LIBRARIES: MANAGEMENT AND POTENTIAL USE

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Sound libraries of time-expanded and frequency-divided echolocation calls are a useful resource for making comparisons between bat echolocation calls from unknown species and those from individuals of known species to facilitate identification. Analyses of calls and statistical treatments have shown that such a comparison of calls can provide a reasonable level of accuracy for separating species or species groups. Individuals who conducted local acoustic surveys for bats wanted reference calls for comparison with other acoustic captures, and were the first to produce call libraries. As the level of sophistication and activity grew, both in the available technology and in applying data about calls to ecological questions, so did the importance of reference materials. Call libraries have expanded to regional and even continental scales, and provide known reference calls covering a variety of geographical ranges. Moreover, as more calls are being recorded, archival requirements have arisen. As with natural history museum collections, calls are being cataloged, archived, and provided to researchers in the same way as museum specimens. In this manner, calls recorded from free-ranging bats can be identified and larger questions regarding geographic or other forms of variation can be addressed. There are problems associated with publicizing call libraries, especially the danger of misuse by untrained users. Misuses include using a visual comparison of a call structure as the only parameter tested, constructing maps of distribution based on unverified recordings, or inferring species absence based on lack of recorded calls. Statistical approaches to using call libraries for species identification are discussed, as are the dangers of pseudoreplication, requirements for estimates of call parameters, and other issues pertaining to call measurement. Finally, we provide recommendations and guidelines for constructing a library of bat calls and for the use or analysis of bat-call data.

Key words: bat detector, call libraries, Chiroptera, discriminant function, echolocation, frequency division, time expansion

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INTRODUCTION

The development of microphones sensitive to ultrasound and apparatus capable of recording high-frequency signals has meant that the echolocation and social calls of bats can be recorded and analyzed. As long as the recordings are not discarded after analysis, they can form a type of archive. Such archives, if properly indexed, form a library of echolocation calls. Once a library exists, a number of questions arise: For whom are the libraries, for what will they be used, how will they be used, and what features of the library are important? The first and second questions address the issue of whether the calls are recorded in the same way that museum specimens are collected; to simply provide a record of a characteristic of an animal, or used actively in research. The British Library National Sound Archive, established in 1969 as the British Library of Wildlife Sound (BLOWS) now holds over 130,000 recordings of animal vocalizations. This collection is among the largest in the world and contains representative samples for many vertebrates. While these recordings provide examples of some bat species, the calls may not provide a statistically representative sample of the full call spectrum of a species or geographical race. So, while this archive is a library in the strictest sense, its ability to provide a resource for analysis of fine taxonomic detail may be limited.

Another call library was formed in 1997 to accommodate a large number of files from the Bat Call Library housed at the University of New Mexico, Museum of Southwestern Biology (http://talpa.unm.edu/batcall/). This call library was modeled after a combination of established call repositories (Cornell Library of Natural Sounds, Ithaca, New York) and another collection of calls for bats (the South-east Australian Bat Call Library – Gannon and Foster 1996)). Libraries fulfill the simple need to properly archive and make available voucher calls collected as a reference set when conducting acoustic surveys for bats in a new area. In the case of the Bat Call Library, one of us (WLG) had been archiving calls since 1993 as part of a statewide survey for bats. Bat surveys require a variety of sampling methods, including trip lines, harp traps, mist nets, and acoustics (O'Farrell and Gannon 1999). Reliable storage and recovery of thousands of call files became a challenge. For most bat researchers, the concept of a call library means that it will be used for resolving biological issues. Some of those issues include matching echolocation call structure to ecology (Neuweiler 1983; Schnitzler and Kalko 2001), exploring taxonomic relationships (Simmons and Stein 1980, Gannon et al. 2001), resolving cryptic species (Jones and Van Parijs 1993, Gannon 1997), or facilitating identification (O'Farrell et al. 1999).

RECORDING TECHNIQUES

Unless the vocalizations of the bats have a frequency component of less than 20 kHz, some form of high-speed data-capture device is required to record the signal. High-speed tape recorders are typically large, making field recordings difficult without a vehicle. The dynamic range or signal-to-noise ratio is often poor, at around 48 dB, and the tape medium is expensive; however, they have high-frequency linearity, can record on multiple channels, and can store a vast amount of data on one tape. Another advantage is that signals can be recorded continuously without any download time. Digital-capture bat detectors sample the input signal at high rates (approximately 450 kHz) and then download those signals to a tape recorder as analog signals at a fixed fraction of that rate. The analog-to-digital (A/D) converter most often works at 8 bits, giving a 48 dB signal-to-noise (S/N) ratio. While such a system is field portable, it has many processes which introduce noise into the system, from the bat-detector microphone itself, to the A/D and D/A converters and the recording system. With a signal-to-noise ratio of only 48 dB, it is arguable whether a good-quality tape recorder is really required for these signals, but poor signal-to-noise ratios in the signal and the recorder may combine to degrade the signal further. The Sony Professional Walkman has a S/N ratio of 58 dB when used with metal or chrome tapes. This gives 10 dB of headroom over the noise level from the detector. Cheaper tape recorders often have no manual method of adjusting signal level and the effect of automatic gain control (AGC) in these recorders is unknown. Generally, needle-based VU meters react more quickly to signal-input level changes than LED indicators and can help to avoid overloading and distorting the resulting signal. However, the limited headroom in the input amplifiers in most bat detectors is more likely to generate clipping than the recorder. Compression algorithms used in mini-disk recorders may also degrade the signal, but the effect of these ATRAC (adaptive transform acoustic coding) algorithms on recorded bat echolocation calls is ambiguous. Portable DAT recorders provide high-quality recordings of analog signals, with a signal-to-noise ratio in excess of 96 dB. They also provide fast indexing of signals, allowing signals of interest to be located quickly. However, tapes are delicate, recorders are sensitive to humidity, and battery life is limited. Direct-to-disk recording minimizes many of these effects by directly sampling the echolocation signal from the bat detector using an A/D card, and then storing it on the hard disk of a computer. Field-portable versions use A/D cards that fit into PCMCIA slots of laptop computers. One extra advantage of this system is that these cards often sample at 12-bit resolution, giving a signal-to-noise ratio of 72 dB, thus extracting the maximum amount of information from the microphone of the bat detector. At 1 MB/s throughput, however, files can be very large and storage becomes an issue. With changes in storage media technology, it may be that direct sampling will replace all the current methods. Direct sampling using a data-acquisition (DAQ) card is perhaps the optimal way to sample echolocation calls assuming that it is able to sample the input signal somewhere between 300 kHz and 600 kHz and direct input to a computer hard drive. Such a system provides instantaneous sampling without the lag time of time-expansion systems and also provides harmonics and amplitude that zero-crossings systems do not provide. Drawbacks to this system currently are: 1) an expensive storage system (approximately US $9,000); 2) large call files (500 kHz sampling rate would require 500 Kb every second or 1 GB every 35 minutes); 3) the need for an extensive database-management system and library; 4) staff to retrieve and analyze many large call files; and 5) the equipment is bulky and fragile, making it currently field incompatible. Of course the same drawbacks held for technologies 30 years ago. Technological advances have occurred to the level where computers (for instance) sit in the palm of the hand. It would appear that direct sampling is the future, and perhaps not too distantly.

Until then, a number of tradeoffs must be tolerated whether employing frequency-division systems such as Anabat that do not record multiple harmonics, intensity, or spectral information, or time-expansion systems such as the Pettersson, which do not allow continuous recording.

As a first stage of generating a call library, the capture and storage of the call is of paramount importance. Good equipment, and above all, good techniques are essential if the recorded call is to be of use for future identification purposes. To maximize the recordings, it is vital to ensure the bat is flying toward the microphone, is in normal foraging flight, that no part of the recording system is overloaded, and that adequate notes are taken about the recording situation.

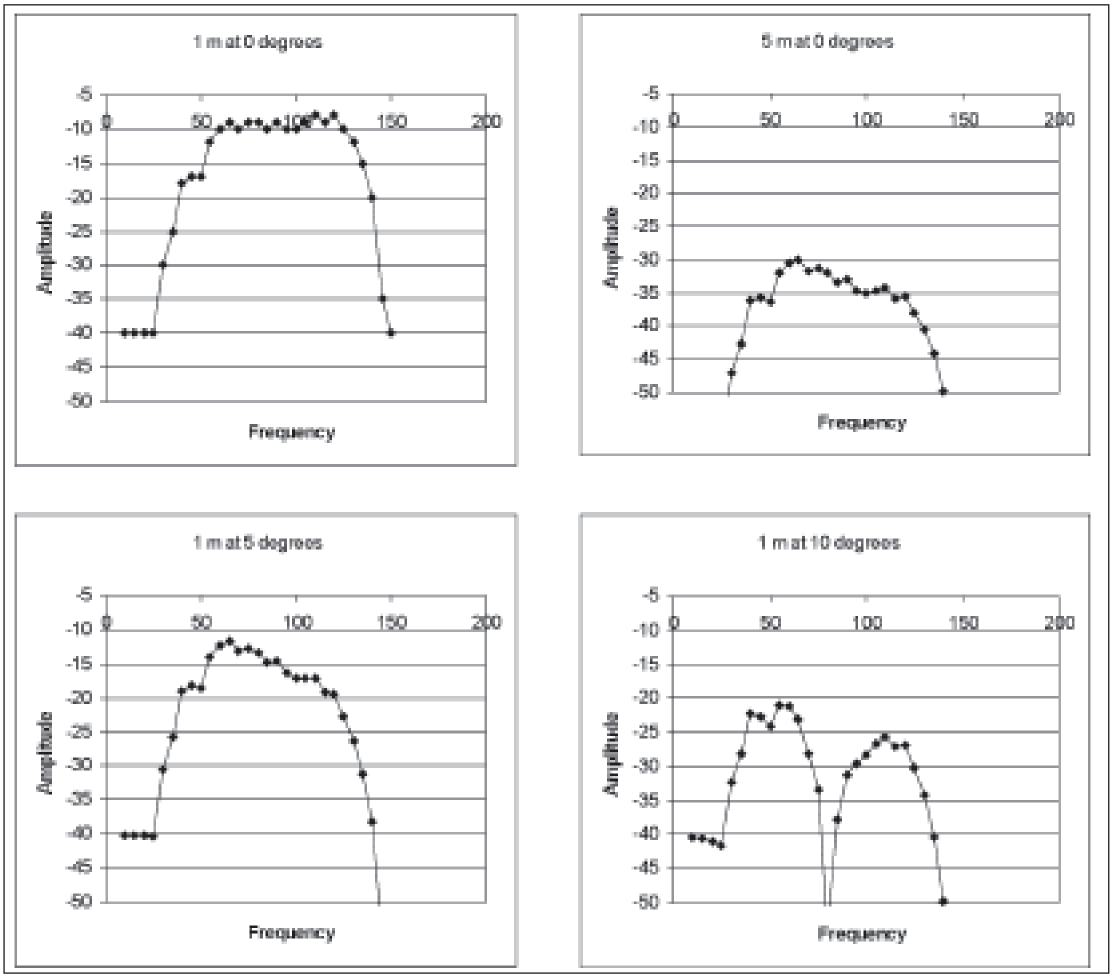
SAMPLING CONSIDERATIONS

For quantitative use, it is important to ensure that recorded calls are a statistically representative sample of the population under study, and at the level required for that analysis. It is also important to define which types of calls or call situations are to be used, i.e., approach calls in open situations. For large-scale issues, a single call may provide sufficient resolution for the analysis, such as relating gross morphology to echolocation call design (Norberg and Rayner 1987). To compare calls from an unknown bat with those of a known species for the purposes of identification, it is necessary to have a statistically representative sample from that species. For the sample mean and standard deviation to be representative of the population mean and standard deviation, at least 30 independent samples are required. Thirty refers to the number of individual bats recorded, not to the number of calls, since 15 calls recorded from each of 2 bats is hardly representative of the species and is a pseudoreplicated measure. Ensuring that calls come from different individuals can be difficult, but changing locations or not using sequential calls will reduce the likelihood of pseudoreplication.

The single most important qualifying factor for the construction of a library to be used for analysis is that the recordings must come from bats that are identified with 100% certainty. In practice, this is not easy to achieve. Bats may be recorded from a roost where the identity of the species has been established from trapping. Alternatively, bats may be captured, identified, and recorded upon release. Both these methods can result in atypical calls, since the bat is not in a normal foraging situation. Bats may be captured, identified, and then light- or radio-tagged and followed for recording. Barataud (2001) used light tags on identified individuals, and recorded them in different foraging situations. Such an approach is commendable but difficult to achieve, especially given the sample size requirements. Even if it is difficult to gain a large sample size using this method, the approach is innovative.

Any intraspecific variability recorded will be a combination of real intraspecific variability (habitat, conspecifics, foraging mode), variability due to the recording process, and variability due to the analysis process. As long as the recording and analysis processes are identical for all calls in the library, this variability will impose no consistent bias. There may be some variation in frequency sensitivity of the recording apparatus, which can have a marked effect on recorded frequency structure, but the greatest source of variance in the recordings is due to the distance and angular relationship between the bat and the microphone. Such variation can be large enough to mask differences among species. One potentially important factor is that in constructiing an analysis library only the best calls should be used. Such calls are identified by a good signal-to-noise ratio and the widest bandwidth in the call sequence, indicating that the bats were flying toward the microphone. While such calls are important in recording the true signal structure, they do not represent the full potential variability of calls recorded in less-favorable situations where there is considerable variability due to distance and directionality effects, and as such may give a misleading sample for comparison to normal field recordings. They also do not take into account the flexibility of bats to produce different call structures in different foraging situations.

Fig. 1 illustrates the effect of distance and directionality on the apparent power spectrum of a simulated broadband FM signal modeled on that of *Myotis nattererii* with a start frequency of 150 kHz and an end frequency of 25 kHz. The same simulated call is modeled at different distances and angles from the recording detector. As com-


1 m at 0 degrees

5 m at 0 degrees

1 m at 5 degrees

1 m at 10 degrees

Figure 1: The effect of distance and angle of incidence on the apparent power spectrum of a simulated broadband FM signal modeled on that of *Myotis nattererii* with a start frequency of 150 kHz and an end frequency of 25 kHz.

monly observed with such broadband calls, the peak frequency is not well defined. At 1 m from the bat and 0º incidence, the full spectrum is preserved. At 5 m and 0º incidence, atmospheric attenuation has removed many of the higher frequencies, lowering the apparent start frequency to 140 kHz but having the greatest effect on the apparent peak frequency, limiting it to the lower end of the spectrum. The effect of increasing the angle between the bat and microphone results in even greater effects. At an angle of 5º, the higher frequencies are lost due to their greater directionality. At angles greater than 5º, frequency notches occur due to side lobes (Waters 2001).

Other less-well-known sources of variability include the variability of the call structure depending on habitat. For example, a bat may use a longer-duration call when foraging in the open than when in clutter. Parameters such as bandwidth and repetition rate are also affected by foraging situation. This can also have an effect on recording variability, since calls may be detected over a greater range in the open and therefore suffer greater atmospheric attenuation, which will low-pass filter the call. Sherwin et al. (2000) classified calls into acoustic guilds (aerial hawking, gleaning, mixed strategist) in an effort to assess differential use of habitat by bats. In that study, bats appeared to "view" the landscape as a large meadow despite structurally complex riparian habitat that remained as a remnant. Calls recorded were predominantly low-to-medium (20-35 kHz) frequency, long-duration calls.

In assessing habitat use by bats, study design is important. Gannon et al. (in press) reviewed 50 papers and found that only 6 explicitly stated assumptions, provided replication, and sampled across spatial and temporal scales. Gannon et al. (2003) concluded that acoustic detectors used alone are inappropriate for investigating ecological questions about bat communities.

STATISTICAL ANALYSIS

The generation of a call library allows for statistical interpretation of data about call characteristics. Simple statistics can facilitate species comparisons and be used to interpret intraspecific variability. However, most often, a library is used to assign a call from an unknown species of bat to a particular species. At its simplest, a visual representation of a call in the form of a spectrogram is compared to a reference call from a known species. Aside from any analytical differences in the generation of the spectrogram of the unknown call and that of the known, this makes no use of any statistical information about the call variability from the population rather than the individual. Such visual interpretation is often used, as it is fast and requires no statistical methods. The major flaw in this method is that there is no estimation of the likelihood of the assignment to a particular species being correct. Some have claimed a high success rate of this method of identifying species (e.g., O'Farrell and Miller 1997, O'Farrell et al. 1999). However, objections stem from the inability for replication by other individuals and the limited training or experience by most who attempt to judge species identifications by the quality of the call alone.

The next level of analysis uses the mean and standard deviations of the library calls as an index for comparison with the same parameters from the unknown call. Such an inspection can be visual or use some basic statistical processing. It is possible to construct a t-score (or Z-score if the number of individuals per species in the library is over 30), which gives the probability of that call parameter belonging to each species in the library in the same behavioral situation. The consensus of probabilities for each parameter in turn can determine the likelihood that the unknown bat belongs to each species. Such a technique violates a number of statistical assumptions. It is a form of pseudoreplication and takes no account of the covariance between call parameters. However, it does provide some indication of the likelihood that the unknown bat belongs to a particular species, as long as parameters are chosen that have high interspecific variability and low intraspecific variability.

The most widely applied technique is discriminant function analysis. In this type of analysis, the parameters measured are evaluated for each species and an algorithm is derived for allocating an unknown call to one of the species groups. Vaughan et al. (1996) used this technique with data about the echolocation calls of British bats and achieved a high correct classification using a cross-classification technique. Such analyses can be difficult to implement or interpret however. The data are likely to violate the assumption that covariance matrices are homogenous, meaning that the computationally easier Fishers linear discriminants cannot be used and the quadratic ones must be used instead. The data are also unlikely to be normal in multivariate space. This is because different groups of bats have different call structures, so that bats using constant-frequency calls, such as *Rhinolophus*, will form a different cluster than *Myotis* since the covariance between different parameters of their calls will be different. In practice these violations may not affect the overall ability of the discriminant function to allocate calls; however, the major disadvantage is that when new calls are added to the library, the discriminant function must be recalculated. While difficult to initially calculate, discriminant functions are easy to use in classifying an unknown bat. For a linear discriminant, the function provides a constant and a series of coefficients for each measured parameter for each species. To classify an unknown bat, it is simply a case of taking the constant and summing up the products of the coefficients and values of each parameter. The value that is greatest indicates the likely species group to which the unknown bat belongs.

For example, consider a discriminant function between two species of *Myotis*:

$C_{species1}$ = $constant_{species1}$ + ($c_{1species1}$ x Fmin) + ($c_{2species1}$ x Fmax)+($c_{3species1}$ x duration)

$C_{species2}$ = $constant_{species2}$ + ($c_{1species2}$ x Fmin) + ($c_{2species2}$ x Fmax)+($c_{3species2}$ x duration)

Where:

C = the classification score

$constant_{species1}$ = the constant derived from the discriminant function for species 1

$c_{1species1}$ = the classification coefficient for species 1 for parameter 1, in this case the minimum frequency

Once the constants and coefficients are entered into a spreadsheet, only the call parameters from the unknown bat need be entered, and the most likely species can be automatically calculated based on which species has the highest classification score.

For analyses where the covariance matrix is heterogeneous, which is likely to apply to most analyses but can be tested using Box's M test (Mardia et al. 1994), these linear discriminants cannot be derived, and a quadratic discriminant should be applied. Classification without using linear discriminants is less straightforward; one technique uses the Mahalanobis distance, which in effect is the 'distance' from the observation to each of the classified groups. The group to which it is nearest is the one to which it is classified. In practice, there may be little difference in classification based on linear or quadratic discriminants, and only verification using cross-classification or jackknifing will show differences. It is noteworthy that if linear classification coefficients are derived for data with a heterogeneous covariance matrix, then there may be a bias in classification to species that show the greatest dispersion (Tabachnick and Fidell 1996). In these cases, a suitable transformation of the predictors should be applied. Two factors that may improve the accuracy of classification are the use of priors and some degree of preprocessing or preclassification. Priors indicate the prior probability that a bat belongs to a particular species group. By default, the priors are equal, that is, each bat has an equal probability of being assigned to each species before the analysis. Such an assumption may not be valid where a discriminant function is derived for all species in a particular country, but the recordings are made in a region where only half those species are known to be present. The priors for those species that are not present will then be zero, and the chance of correct classification of the unknown bat will therefore be improved. This can, of course, turn into a circular argument in that the purpose of the analysis is to establish the range of species which may occur, but which are being excluded from the analysis because they do not occur.

Preclassification may be of use when calls from the range of species to be discriminated fall into a number of discrete categories that are so clear as to have no need of discriminant function analysis. Such a division may occur between bats using FM calls and those using CF calls, or where a single bat emits two or more different call types. The construction of two discriminant functions, one for each group, may improve the accuracy of the discrimination within each group, and may also help to alleviate the problem of heterogeneous covariance matrices.

ARCHIVING AND DISSEMINATION

The degradation of analog recordings with time is a serious issue. Most recordings of bat vocalizations are still made using some form of analog tape, either directly in the form of high-speed tape recorders, or as an analog output after digital capture. Analog tapes decline in quality even when stored at optimal temperatures and humidity and away from magnetic sources. The advent of cheaper digital-storage media now means that it is possible to permanently archive recordings onto compact disk or DAT tape, although the long-term stability of DAT is still in question. As discussed earlier, the ATRAC compression algorithms used in minidisk recorders affect the quantitative analysis of calls, but the magnitude of this effect is unknown and may be within acceptable limits. Digital storage is clearly the best mechanism for long-term archival of signals. Not only does it ensure that there is no degradation through time, but also long sequences of signals can be edited and indexed for fast retrieval. Digitizing analog tapes is probably most often performed using a PC rather than a tape-to-CD-writer setup, as this allows editing of the signals. Even for bats using lower echolocation call frequencies, it is beneficial to use the highest sampling rate available, usually 44.1 kHz for most soundcards at 16-bit resolution. The connection of line-out to line-in sockets is much better than headphone-to-microphone inputs as it will degrade the signal less and is not dependent on the volume settings on the tape device. As there is no functional difference between the files stored as audio tracks on a CD and those used by the PC, signals stored in this way can be made available via the Internet for collaborative study. The default setting for audio .wav files is not compressed, and therefore, there is no loss of quality in the production of the file, but they are necessarily large. Recording in mono will halve the file size of a stereo recording if only one track of data is required. One minute of mono data recorded at 44.1 kHz and 16 bits will occupy about 5.2 Mb. Transfer over a standard 56 kbps dialup Internet line would take over 12 min. Compressed formats of Internet audio transfer such as Real Audio and MP3 compress the signals using a lossy algorithm rendering objective analysis impossible. If data are to be shared from a library in this way, the nature of the library, as well as its purpose, must be defined. Data can be provided in its raw format as .wav files, a sub-sample of the best-quality signals can be provided for reference, extracted parameters from the calls such as duration, peak frequency, etc., can be made available, or sample sonograms and power spectra can be provided.

A bat-call library was constructed at the University of New Mexico in response to a perceived need for a central distribution site for reference, research, and exchange of calls recorded from bats using the Anabat hardware and software (Fig. 2). This library has two main functions; one for general reference and the second as a true collection of calls modeled after natural history museum curatorial practices. The reference section consists mostly of species accounts. Species accounts have a standard format of at least one photograph of a living bat, a series of .wav files, and a representative spectrogram generated from one of the call files in the call library for that species. Standard information on taxonomy, identification, ecology, and behavior of each species follows. Other sections include a link to other sites concerning bat biology, news about the development of the site, recently developed software for download (although the most recent Anabat software updates can be found at *www.hoarybat.com*), and a listserv where bat enthusiasts can participate in discussions about bat acoustics.

The database was the most problematic part to develop. Anabat-generated call files have an unconventional file extension. Standard file names are 8 digits that describe the date and time when the call was recorded. The file extension consists of 2 more numbers and ends with a "#" symbol (i.e., 12345678.99#). The "#" is a null symbol for DOS-based systems. Posting call files with this extension is not a problem, however download protocol prohibits the use of the "#". Therefore, we produced a program using Winzip to put requested files into the Zip format before downloading proceeds. Another alternative is to convert Anabat files to .wav files using the Anamusic program. However, the files then become unusable for display or analysis by Anabat programs. Call files could be renamed excluding the "#" and then exported, but that extension symbol must then be added before running the call file on the client machine. Until Anabat is reassembled in a Windows-compatible format, the download utility developed for the bat-call library must be used to acquire call files. Uploading or posting calls on the Web must be accomplished by sending call files via email to the Web administrator. Anonymous FTP and other methods that give direct access to the library pose a significant risk of sabotage. Routing calls to a site administrator slows the posting of calls to the Web but should increase the quality of postings.

Call files that are archived need to have the following standard header information: collector name and email address, date or season of collection, taxonomic information (genus and species), specific location of the recording (general to specific starting with country, state, county [only useful for the United States]), and specific locality. Although no GIS component is currently operational, standard coordinates (such as latitude, longitude, and elevation) are encouraged for all material accessioned into the library. Call metadata is also encouraged that describes the habitat, conditions, and equipment used during the recording session.

A prototype North American Continental Bat Acoustic Monitoring (CBAM) program was begun in 2001 with the intention of placing Anabat-based acoustic monitoring stations in a grid across the continent, mostly at biological field stations. Continued technical advances have delayed aggressive deployment because it now appears that a bat-call detector can be placed in conjunction with a "smart" interface module, compact-flash data card, and battery pack, all in a volume the size of a small lunchbox (such a unit by Compaq currently costs approximately US $800). These units need only be checked monthly. Other possibilities for these units include detectors tied to cell phones that can be called for data collection for posting on a website for analysis. At this point, 2 units are deployed in New Mexico, 3 near Las Vegas, Nevada (M. J. O'Farrell, pers. comm.), and 1 at the Point Reyes Bird Observatory in Cal-

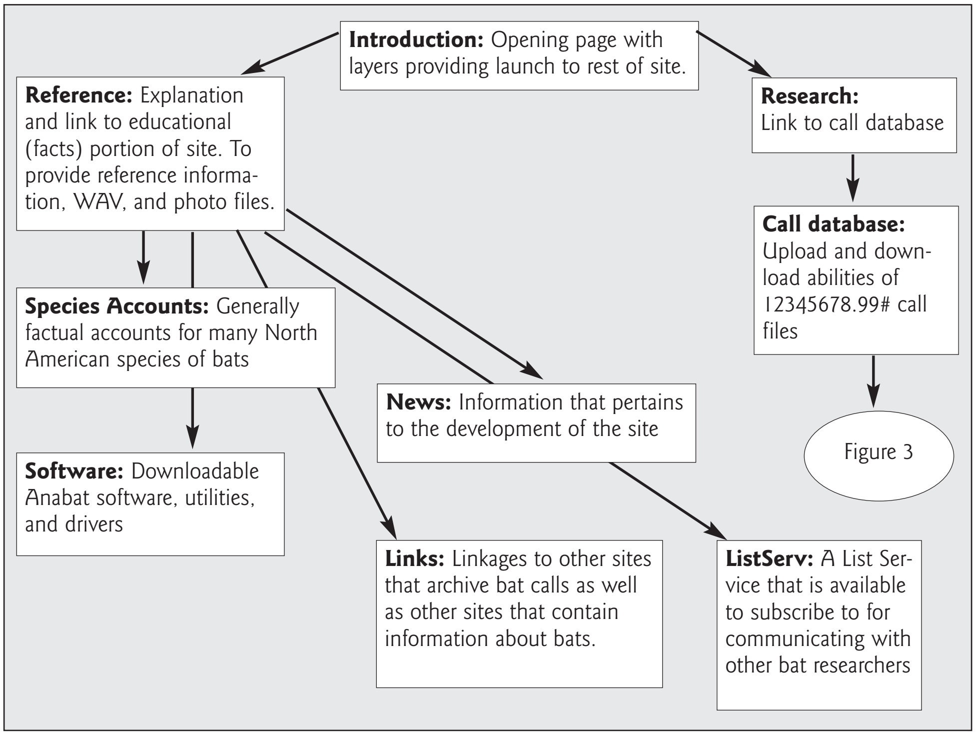


Figure 2: The page structure of the bat-call library at the University of New Mexico consists of an introductory page, species accounts, reference, research, news, a listserv, software, and links.

ifornia (C. Corben, pers. comm.). Although none of these 3 sites are currently linked, they represent a test phase to improve the design, reliability, and application for a continental network. When more resources are applied to this effort, it is hoped that CBAM will provide long-term, large-scale data about bat species across a variety of habitats.

RECOMMENDATIONS

Calls of free-flying bats recorded during a survey should be compared with a call library constructed from recordings made after capture and release of bats from the same general area of the survey. Some problems may be experienced with high-flying species that may take some time to produce normal search phase calls after release. In these cases, species-specific protocols for the collection of calls may be needed to ensure that the library will match calls recorded during a survey.

Assumptions underlying the study design of any acoustic study should be clearly articulated prior to data collection and specific protocols should be published and adhered to.

Calls should be collected in a standard fashion described in the study's materials and methods section.

Variables used for analysis of calls should be clearly defined.

Calls used for analysis should be edited in a standard, stated fashion before analysis. If any filtering has been applied, the precise nature should be stated, and unfiltered calls provided as well.

Analysis should fit the study design and the purpose of the data collection. Simple descriptive statistics may be sufficient at times. Principle component analysis should be conducted when no known pattern exists (i.e., where there is no species list). Discriminant analysis, classification trees, or other multivariate classification techniques should be employed using a training dataset prior to running test data.

All calls should be archived in their raw form with complete and accurate metadata in a bat-call library. Calls represent vouchers and as such are original data that should be cataloged and archived.

Bat-call libraries should be generally available to any qualified user and follow the use and management procedures of natural history museum collections.

Publication rights, intellectual property rights, and authorship rights should be explicitly stated.

SUMMARY

We have summarized the current status and value of a bat-call library. Although there are several different foci that libraries are taking (analysis versus archival), libraries serve as a base for acoustic study. Whether born out of a need to archive or analyze, libraries are part of a natural progression for a discipline that has matured and gained general acceptance. When first developed, acoustic detectors were a small part of a larger research effort by a few scientists. In the last 10 years, technical advances have allowed a more general application to ecological questions. We feel that libraries are essential to providing a reliable source for information about call data. Increased collaboration and standardized data collection and analysis are essential if bat-call libraries are to be of use for the study of bats in the future.

Museum of Southwestern Biology, Bat Call Library

Figure 3: Updated monthly, the University of New Mexico database call files can be queried either taxonomically or geographically. Searches can also be done by field author, year, season, and call-library number. Query results can be zipped and downloaded.

ACKNOWLEDGMENTS

We wish to thank the many students, technicians, and volunteers that have helped over the years to collect and maintain our respective call libraries. Funding support for these activities has included the National Science Foundation, Undergraduate Mentoring in Environmental Biology program (DEB-0102773), the National Aeronautic and Space Administration, SharpPlus and Pursue programs (PP-114-01SU), and the general support of the Museum of Southwestern Biology, Division of Mammals, University of New Mexico (T. L. Yates). In particular WLG thanks L. T. Arciniega and N. M. Gannon for inspiring this evaluation of life. DAW thanks Nancy Vaughan for the use of her recorded material.

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DESIGNING BAT-ACTIVITY SURVEYS FOR INVENTORY AND MONITORING STUDIES AT LOCAL AND REGIONAL SCALES

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Many bat studies have incorporated surveys using bat detectors to assess the presence and/or activity, or changes in relative abundance of bats, usually detected by counting "bat passes." The design of these studies, the field methods followed, and the data analyses used have differed widely among studies, often due to different aims or practical limitations. Most frequently, the number of people and their experience, and type and number of bat detectors available affect the basic design of studies. However, when the aims and practical limitations are set, decisions still need to be made about the type of sampling method, the size and number of sites surveyed, the deployment of different detector types, and timing of surveys (when and how often to survey for bats). In this paper, we examine different bat-detector survey approaches at local and regional scales and consider analysis tools appropriate for aiding survey design.

Key words: bat surveys, echolocation, monitoring bat activity, power analysis, survey design

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INTRODUCTION

Methods to survey and monitor bat populations are facing increased scrutiny by researchers and biological resource managers nationwide (Hayes 2000; Miller et al. 2003; O'Shea and Bogan 2000). There is growing recognition that while advances in bat-detector technology provide an opportunity to expand our knowledge about populations, there is a need for clear guidelines on how to design standardized bat-detector surveys to provide statistically defensible answers to questions with widely varying purpose and scale. Methods that attempt to standardize the way surveys of local species are approached can advance our ability to understand habitat needs, species' distribution patterns, and population trends at regional and potentially national scales (e.g., Jaberg and Guisan 2001; Macdonald et al. 1998; Walsh and Harris 1996a).

The majority of bat-detector surveys are tied directly to conservation management questions. The simplest survey aim is to determine what bat species are present or absent in a given area. Such surveys relate the distribution of species to land use and provide a means of assessing the relative value of landscape areas for bats. The surveillance of species' distribution over time can be used to monitor temporal changes in the ranges of populations and their use of habitat. A second aim is to determine where bats are feeding and commuting in a given landscape. Surveys to locate key sites and foraging areas for special protection and to quantify bats' use of different habitats provide important information for the development of habitat-management guidelines. Such surveys can also answer specific questions about the ecology of the study species, such as seasonal activity patterns. A third important aim is to track how bat activity changes at sites over time, allowing researchers to identify potential declines and threats to populations at an early stage.

The simplest sampling design is an array of sites at which measurements of activity are made within and across years. Basic design choices include the number of sites sampled, when and how often a site is surveyed within a year, and the duration of each survey and of surveys across years. Standardization and consistency in the application of survey protocols are key elements in designing surveys that are to be extended to longer-term monitoring if results are to be reliably compared over years. In addition, estimating the natural temporal and spatial variation in the bat-activity measure (bat passes) and variation introduced by the measurement process is an important part of evaluating study design. While a strong emphasis should always be placed on the statistical design of surveys and monitoring schemes, there is the need to seek solutions that balance statistical assumptions with the practical and logistical demands of field-based schemes.

The selection of survey design is affected by multiple interacting factors. Ideally, the process should involve the following steps:

- Define the scope: identify species and populations of interest
- Define the purpose and scale: state the questions/hypotheses/aims
- List the logistical constraints
- Adjust the questions/hypotheses/aims if necessary/permissible
- Select field techniques and a suitable sampling design and protocol to minimize bias and maximize accuracy and precision.

DEFINING THE SURVEY'S SCOPE: IDENTIFYING SPECIES AND POPULATIONS OF INTEREST

Knowledge of the ecology and behavior of the species to be studied is the first step in planning a survey, and will dramatically affect the choice of survey design. This includes any prior knowledge of roosting habits, foraging behavior, seasonal movements, and how environmental factors may affect activity and local abundance. Bats are a diverse group of mammals in terms of ecology and will often require the application of multiple survey approaches. For some species, the use of bat detectors may not be appropriate (e.g., calls are too low in intensity to be detected easily or the animals fly too high to be detected from the ground). A review of the current scientific literature is vital prior to planning a survey.

When target species have been selected or information about the species expected to occur in the survey area have been listed, then a consideration of their ecology is warranted. While some species are colonial and gather in huge colonies at well-known underground sites, others are widely dispersed, roosting cryptically in foliage, tree cavities, and rock crevices. Detection probabilities therefore differ between rare and common, restricted and widespread species, and among landscapes. Further, the varied ultrasonic repertoire of bats is related both to the species and the type of environment in which they are flying. Some species can be readily identified from echolocation calls using a bat detector; others can only be identified in favorable situations, with considerable experience or by computer analysis; and for some species, identification success rates are low even when using the most advanced analytical techniques (e.g., some *Myotis* spp.).

Echolocation calls are produced to perceive the environment, and the nature of the environment dictates the type of call that is produced. This contrasts with bird song (Barclay 1999), which is a repetitive series of notes sung irrespective of the environment the bird is found in. For example, bats in open areas produce loud sounds that travel long distances, whereas the same bat in cluttered habitat may produce quieter sounds to minimize listening to non-target echoes (most bats avoid pulse-echo overlap to minimize self deafening, irrespective of call intensity). Our ability to detect most bat species has not been measured accurately in the field because of the difficulties in doing so, although some attempts have been made (Forbes and Newhook 1990; Waters and Walsh 1994). Recent developments in equipment to broadcast simulated echolocation calls of different species may enable experiments to measure detectability with more precision (G. McCracken, unpubl. data). Differential detection rates among species mean that comparisons of abundance cannot be reliably made among species and can only be made if data are weighted for differential detectability. While it is generally assumed that detection rates remain constant within a species, making possible an evaluation of changes in abundance, differential detection rates among habitats (open, edge, clutter) may confound such comparisons if the habitats sampled differ dramatically in structure (Parsons 1996; Patriquin et al. 2003). For example, if repeated monitoring of a location is carried out over time in which the area of cluttered woodland declines and becomes fragmented, sampling of the same sites may lead to the erroneous conclusion of an increasing population if detection of more bats forced into open/edge situations occurs. Knowledge about the potential magnitude of this bias is essential. Generally the assumption is made that the magnitude is small, since many species appear to show habitat preferences for either open or edge/cluttered habitats and their flight maneuverability may restrict them to these habitats (e.g. *Nyctalus noctula*, a fast-flying species with narrow wings, is rarely found in cluttered habitats). However, bats can exhibit surprising flexibility in their habitat use in different landscapes. *Myotis emarginatus*, a gleaning bat generally considered to hunt in dense tree crowns, was recently recorded hunting over open salt marshes in western France (H. Limpens, pers. comm.).

DEFINING THE PURPOSE AND SCALE OF SURVEYS: STATE THE QUESTIONS/HYPOTHESES/AIMS

An important factor to consider at the outset is the purpose and scale of the intended study. Appropriate methods for a particular study become more obvious if there is a clear goal, specified in advance. Most studies are tied directly to conservation-management needs and often have multiple but interrelated objectives. The most basic survey objectives include the need to detect the presence/absence of bats or the activity level of bats – also referred to as the index of abundance or relative abundance. Key sites and feeding areas may need to be located to provide information pertinent to local site management. Often, the objective of the survey is to provide data about specific species, and thus the need for species' discrimination is introduced. Frequently, managers are required to inventory (list) the species present in an area, or to identify habitats used/not used by each species or by a species of special interest. A more demanding objective is to determine the relative activity or abundance of species and identify the most preferred or avoided habitats. Finally, the objective might be to monitor changes in distribution or relative abundance to ascertain patterns or trends over seasons or years.

LOGISTICAL CONSTRAINTS

Once the general objectives and scope of the survey have been defined, the next important step is to list the logistical constraints and adjust expectations where necessary. Often, logistical constraints will predetermine the end objectives and scope of the survey.

Observers

The number of skilled observers available to operate technical bat-detector equipment will constrain the number of sites which can be visited within a time window. A volunteer force of trained observers operating low-cost, easy-to-use equipment is one way that large numbers of sites spread across a large region can be surveyed (e.g., Limpens 1993; Walsh et al. 2001). However, this will introduce higher levels of observer variability. Examples where this approach might be used include: where the aim is to quantify the presence/absence or abundance of bats without a requirement for species identification; in regions where only a single species is present (e.g., Hawaiian hoary bat, *Lasiurus cinereus*); where more than one species is present, but they are easily distinguished (McCracken et al. 1997; O'Donnell 2000); or where one species has highly specific calls and can be distinguished with confidence from others (e.g., *Euderma maculatum* in western North America; Fenton et al. 1983).

Equipment

Whether or not more than one system is available, you can still choose to survey one or more than one site per night.

Money and time

Costs and the availability of funding and time will always be key limiting constraints.

SELECTING FIELD TECHNIQUES

Bat Detectors and Quantifying Bat Activity

A range of bat detectors are available to conservation biologists and professional bat researchers at reasonably affordable cost. Although these are described in detail in other chapters of this volume, we briefly summarize them in relation to practical field use. The transformation of ultrasound into audible sound or visual depictions can be made with one of three main types of detector: heterodyne, frequency-division, and time-expansion. The choice of a bat-detector system depends upon the planned use for it and budget.

Heterodyning and frequency-division are real-time methods, and thus observers can hear the sound from the detector at the same time it is emitted by the bat. Heterodyne detectors are sensitive, cheap, and simple to use, but require tuning to a specific frequency. Only frequencies within a bandwidth of approximately 10 kHz of the tuned frequency will be picked up by the detector (i.e., tuned to 45 kHz, frequencies from 40-50 kHz will be detected). Note that different detector types have different bandwidths (Waters and Walsh 1994). This limits the ability of a heterodyne detector to detect all species when the bat assemblage includes species with widely separated call frequencies. In certain species-assemblage scenarios, however, all species may have some element of their call that sweeps through this frequency range. The resultant sound from a heterodyne detector can have tonal qualities such as "dry ticks" and "wet smacks" which relate to the type of ultrasound the bat is producing and, combined with rhythm, repetition rate, and frequency characteristics, can form the basis for identification (e.g., Ahlen 1981, Limpens this volume). For many species, calls are difficult to separate by ear without extensive training. Species may be considered together, or grouped by frequency or genus, to avoid bias introduced by unsuccessful identifications. This may provide information at a satisfactory level of detail in many survey situations. Alternatively, identification success rate can be quantified, and if an acceptable level is reached (e.g., 90% success rate), identifications to species can be made. Identification to the species level will undoubtedly be most accurate when only a single species is present, or when a species has unique call characteristics that definitively separate it from all other species present.

Frequency-division and time-expansion detectors transform the entire ultrasonic frequency range without tuning (i.e., they are broadband detectors that detect all frequencies), and in this regard, they are considered better suited for multi-species surveys. Frequency-division detectors reduce call frequencies by a specific factor, are medium in price range, and allow rapid visual identification of some calls using a portable computer in the field by comparing calls with known-species calls from a library. Calls can also be recorded to a tape recorder or memory card, and call parameters measured later using sound-analysis software. With time-expansion detectors, calls are generally recorded and identified through the use of sound-analysis software, although some researchers in Europe have developed skills to identify time-expanded calls to species (Barataud and Tupinier 1999). Time-expansion detectors are less suitable for carrying out real-time surveys that aim to estimate bat abundance, because calls will be missed during the time it takes to download recorded calls to tape. They are expensive, but the transformation of call information is more complete, making them suitable for studies where discrimination between similar species is of prime importance. The problem of species discrimination for some species (e.g., *Myotis* spp.) is not eliminated, but confidence limits on the level of accuracy are improved.

To assess activity with a bat detector of any type, different sample statistics may be recorded. Most frequently, the number of bat passes per unit time or per distance traveled by an observer on a transect is recorded. A bat pass is defined as a continuous series of echolocation calls heard on a bat detector as a bat flies within range (Fenton 1970). The number of bat passes in a specified time period or distance is counted as an estimate of bat activity. Other options include recording the length of time filled with activity, the time elapsed before a bat is detected, and the number of feeding buzzes. "Feeding buzz" describes the high pulse-repetition rates associated with attacks on prey by insectivorous bats (Griffin 1958). The number of feeding buzzes recorded when bats are active in an area may be correlated to the number of bat passes (unless the site is used by commuting bats), although feeding buzzes will be fewer (Furlonger et al. 1987; Walsh and Harris 1996b). It is usually only valuable to analyze feeding buzzes separately if the aim is to identify feeding "hotspots" or investigate feeding behavior. By recording the number of feeding buzzes as a proportion of passes, an estimate may be gained of foraging attempts per unit time searching for prey or commuting. Activity may be averaged over the night, or over a certain portion of the night, and recorded in different habitats. Data can be pooled for all species or separated by species and can be pooled for all habitats or separated by habitat. The more you subdivide the sample, the more likely you are to have zeroes in the data set, and thus the more likely the data will not closely follow a normal distribution. This will make statistical analysis less robust. In areas of high activity, different observers may over- or under-estimate the number of bat passes, thus some randomization of observers among sites is valuable.

Bat detector deployment

Awareness about the level of sensitivity of different bat detectors is helpful. Detectors of different type and manufacturer vary in their level of sensitivity (Fenton 2000; Waters and Walsh 1994), and variation between detectors of the same make has also been noted (Forbes and Newhook 1990; K. Livengood, pers. comm.). Accounting for slight differences among detectors is best achieved through randomizing the placement of detectors and ensuring each detector is calibrated prior to the start and during surveys (Thomas and West 1984). Batteries should be fresh, of the same type, and replaced regularly. O'Donnell (2000) demonstrated that the number of bat passes recorded by the Stag Bat Box III is reduced by 50% after 20 hours of using the same batteries.

Detector systems monitored in person

- The detector is moved more or less continuously along a "line transect" of set or variable length through a variety of habitats or the same habitat (e.g., Barclay 1985, 1991; Walsh et al. 2001).

- The detector is kept stationary at "points" either in the same habitat or a variety of habitats. Points may be placed equidistantly along a transect of set or variable length, or randomly placed within blocks (e.g., Verboom 1998; Hogberg et al. 2002).

There are several advantages to carrying out point counts rather than line transects. Although a smaller area is sampled, the observer does not have to negotiate possibly difficult terrain in the dark, and problems associated with ultrasound created by movement through the environment are eliminated. Additionally, survey design is easier to address, habitat is easier to record at a point than along a line, and multi-species surveys might be easier to conduct. However, transects allow more sites to be surveyed in a given time period even when equipment is limited. This can control for environmental variation from night to night and also reduces the possibility of making multiple recordings of the same bat.

In deciding between a line-transect or a point-sample scheme, it is important to appreciate the potential biases that arise by following paths or other linear features when conducting transects. If, for example, observers tend to walk along woodland edge, rather than following the idealized route through the woodland, then data about habitat use will be biased in favor of woodland edge. Further, if there are density-dependent effects on habitat use, estimated trends in abundance will be biased. If river or woodland edge is a favored habitat, and bats only use more marginal habitats when densities are high, then transects along the favored habitat may show little evidence of a trend in numbers during a period when trends in marginal habitats are large. Thus there might be no observable decline in the favored habitats during a period of substantial decline in overall numbers.

Detector systems set up in situ and activity monitored remotely

- Systems are deployed at different sites of the same habitat type.
- Systems are deployed in different habitat types.

Several systems have been developed by researchers to monitor activity remotely (e.g., Crampton and Barclay 1998; Downs and Racey 1999; O'Donnell and Sedgeley 1994; Patriquin and Barclay 2003; Walsh 1995). Remote monitoring allows personnel to do other activities, such as mist netting, radiotracking, etc. It also means that long hours of work at night are not necessary. However, in-person monitoring may allow individual bats to be followed with the detector, thus providing better quality calls for identification, and depending on light conditions, visual observations may aid species identification. The air space can be scanned with the detector thus increasing the number of bats detected.

When multiple systems are employed, orientation and height above the ground of the detectors should be the same (e.g., 45° to the horizontal, 1 m off the ground). Rotate where each system is used. Do not always use unit 3 in habitat 1, for example.

SURVEY OPTIONS: SAMPLING DESIGNS AND PROTOCOLS

Temporal and spatial variance in activity: sampling allocation and effort

Bats have distinctive activity patterns, both seasonally and nightly. Their activity is strongly influenced by time of night and prevailing weather conditions (e.g., Hayes 1977). Many species exhibit bimodal nightly activity patterns, with a large peak at dusk and a smaller peak at dawn (e.g., Kunz 1973; Maier 1992). Thus, conducting surveys for about two hours after dusk is likely to produce the maximum encounter rate. However, peaks in activity may vary over time or among species, and thus activity or species recorded during only a portion of the night may not accurately reflect overall activity or the total bat community (Hayes 1977). Recording activity throughout the night, through the use of sound-activated or continually recording unmanned systems (e.g., Downs and Racey 1999) is one option to accomplish full-night surveys when personnel is limited. The variation in ambient conditions should be controlled for if possible, by measuring appropriate variables (e.g., air temperature, precipitation, wind strength, moon phase). Ideally, measurements need to be made at each site on a given night, as these may vary from site to site. Qualitative or quantitative measurements can then be incorporated as covariates and controlled for in appropriate models. However, if the technical capacity exists, paired sampling of sites is a more satisfactory way to control for night-to-night variation in ambient conditions (e.g., Vaughan et al. 1996). In addition, it may be necessary to repeat sampling at the same study areas for several nights, preferably under similar conditions. Not all nights will be suitable for sampling due to weather constraints (e.g., Grindal et al. 1992), and additional nights should be factored into any study design.

Activity also varies spatially (i.e., vertically and horizontally) within and between habitats and locations (e.g., Sherwin et al. 2000). An efficient strategy is to apply some form of random-stratified sampling in the selection of specific sites and habitats to be sampled. This can be an integral part of the sampling strategy or can be applied after the sample has been collected using appropriate criteria (Cochran 1977). For example, a study area may be divided into six key habitat types, and samples of sites apportioned proportionately to habitat availability (e.g. Walsh et al. 2001). Vertical stratification of activity (Kalcounis et al. 1999) is especially difficult to measure or account for because detectors are generally placed at or near the ground.

Assessing and monitoring bat presence and/or species presence at single and multiple locations

Are bats present or absent in a given study area? What species (or species groups) exist in a given study area? Which habitats are being used by bats in a given study area? Which habitats are being used by specific species (or species groups) in a given study area? To address questions without species identification, the use of heterodyne detectors is a low-cost option to identify presence or absence of bats. A simple design might be to divide the study area into key habitat types and sample each habitat type for a set time in rotation (e.g., Barclay 1990; Walsh and Mayle 1991).

In a night, should replicates of the same habitat type be surveyed, or one of each habitat type? Over the study, should you survey at each site once and maximize the number of sites of each habitat type, or collect data on multiple nights at the same sites? If the question is about habitat types, then the more sites of each habitat type the better you will be able to generalize across sites within each habitat type and control for spatial variation (i.e., better to have more sites than more nights per site). If your question is about specific sites, then surveying each of those sites a number of times will help generalize across time periods and control for temporal variation.

A common question asked by researchers planning to inventory species at a site is: How long must I survey before I can say a species is not present? The construction of species' accumulation curves (plots of numbers of species recorded versus number of sampling nights) for different habitats is needed to determine the minimum effort required to reach complete inventories. Moreno and Halffter (2000) calculated species-accumulation curves for bats in Mexico based on mist-netting data, and estimated that 5-18 sampling nights were required to reach an acceptable level of efficiency (90%) in inventories within each vegetation type. Interestingly, a similar study in California also achieved annual species assemblages after 6-19 nights of effort, and it took 6-11 nights to capture the six most common species present in the area (Weller and Zabel 2001). The time required will vary depending on the relative rareness of species.

By extending inventories and assessments of bat presence or absence over time, local and regional species diversity can be monitored. On a regional scale, it should be feasible to monitor distributional range shifts by species, although most atlases currently produced do not use bat-detector records as valid records. There is a need for review of quality guidelines and storage of species records made using bat detectors.

Estimating and monitoring bat abundance and/or species abundance at single and multiple locations

Does a given species use one study area more than another? Are there relatively more individuals of a given species in specific habitats? Does the relative abundance of a species using a study area differ over time? Habitat selection and spatial variation in abundance can be determined by assessing activity across a landscape. By monitoring changes in relative bat abundance over time, temporal changes in activity patterns can be assessed and population trends identified.

Estimates of bat abundance (activity counts) using bat detectors are indices of population size, not absolute measures of abundance or density. To monitor changes in relative abundance, it does not matter if it is impossible to determine whether a count of five bat passes corresponds to five different animals or to one animal passing five times. Provided the average number of passes per bat does not change over time, the number of passes can be taken as an index of the number of bats. If the number of passes declines by 50% over a 5-year period, and other factors remain unchanged, then we estimate the number of bats has halved. This, however, necessitates the following assumptions:

A) There is no trend over time in the sensitivity of the equipment.

B) There is no trend in detectability of bats over time.

C) Detected passes are reliably identifiable to species. Alternatively, a proportion of passes is identifiable, and there is no trend over time in this proportion.

D) Transect lines are placed randomly. Failing that, they might be placed in the same, non-random locations each year, in which case trend estimates will apply to the locations covered, and not necessarily to a wider area of interest.

Perhaps the most problematic requirement is the first; as technology improves, it is difficult to justify retaining inferior equipment. If better detectors are introduced, calibration will be required for each species against the old detectors. Alternatively, if a measure of the effectiveness of a detector can be recorded, the analyst can adjust for it. However, such sequences of data are notoriously difficult to model reliably.

In areas of high activity, it can be difficult to count

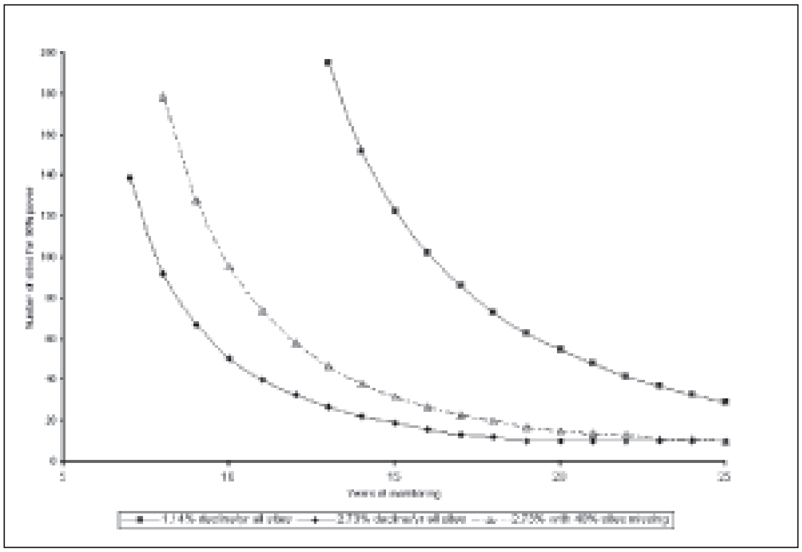

Figure 1: Relationship between number of years of monitoring and minimum sample size needed to achieve 90% power to detect existing declines of 1.14% and 2.73% per annum in pipistrelle bat populations in the United Kingdom.

the number of bat passes in real time. If observers cannot reliably estimate the number, there is the potential for bias in estimated trends. It does not matter if the counts are subject to error, provided that observers do not consistently under- or over-estimate the number of passes. If, for example, there is a tendency to underestimate the number of passes at high densities of bats, then any decline in numbers of bats would also be underestimated. Experiments with independent counts by different observers may prove useful for assessing observer ability to estimate number of passes in this circumstance. Example studies that have monitored abundance over time are few (De Wijs 1999; O'Donnell 2000; Walsh et al. 2001). In contrast, there are many example studies comparing bat activity between areas or among habitats (e.g., Crampton and Barclay 1998; Law and Chidel 2002; McCracken et al. 1997; Patriquin and Barclay 2003).

Power analysis as a design tool for long-term monitoring programs

Developing statistically defensible protocols for monitoring trends in animal and in particular bat populations over the long term is particularly challenging (Macdonald et al. 1998; O'Shea and Bogan 2000). Variability of counts of bat passes associated with time, weather or sampling error can cloud the identification of real changes in abundance. The ability of sampling regimes to correctly pick out significant changes in abundance once all other factors influencing counts are taken into account can be estimated in the planning stages of a monitoring program using a power analysis.

The risk of not detecting a trend in a population when one actually exists is a statistic known as "power", and is most frequently expressed as percentage. If a program has 90% power, this means the statistical power of the monitoring program is 90% to detect a population trend of a specified magnitude. If the power of a monitoring program is below 80-90%, population declines may go unrecognized, and researchers run the risk of wasting resources from the outset because the sampling regime may be inadequate to detect trends with confidence. Power is influenced by interactions between sample size (number of counts), duration (years of monitoring), frequency of surveys, the magnitude of change (trend) in the population over time, and the ability to control stochastic variability of counts because of other factors (e.g., weather). In a power analysis, the effects of these sampling design choices on the power of a program is assessed using count data, and this provides figures that can be used by conservation managers to select options for designing programs. Ideally pilot data collected over a minimum of two years is required. Options for power analysis methods are complex, and advice on determining an approach that fits your needs and data should be sought from a statistician (see a discussion on the Patuxent Wildlife Research Center web site: *www.pwrc.usgs.gov/resshow/droege3rs/salpower.htm*)

An analysis conducted by Walsh and Langton (unpublished data) using bat count data collected for a survey of 45 kHz pipistrelle bats (*Pipistrellus pipistrellus*) in the United Kingdom illustrates some general design choices and their effect on power. Counts of bat passes using heterodyne bat detectors were made on two within-year visits to multiple survey transects. In this analysis, random variation was calculated within and between transects and years from pilot study data using Residual Maximum Likelihood (Robinson 1987), and a route regression technique was applied incorporating individual error terms to estimate power (Sauer and Droege 1990). Sample sizes (number of transects) needed to achieve 90% power over a 5 to 25 year monitoring period to detect either a 1.14% annual decline (=25% cumulative decline over 25 years) or a 2.73% decline (=50% cumulative decline over 25 years) are shown in Figure 1.

Greater sample sizes are required to detect smaller annual declines. In addition, the greater the sample size achievable, the greater the power to detect trends over shorter time frames. Longer survey durations, e.g., over 10 years, permit a reduction in sample size to achieve the same level of power and enhance the ability to detect smaller trends. In addition, a greater sample size is required to offset the reduction in power caused by the inclusion of missing samples over time. While not demonstrated in Fig. 1, greater precision in evaluating an average trend over time is achieved by increasing the number of sites rather than by allocating additional within-year visits to individual sites (e.g. Larson et al. 2001). The confidence in such results for smaller-scale studies will undoubtedly increase with repeated sampling however. A major point of consideration is the dominating effect of time over these survey variables, when conservation objectives are most often time limited.

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CONCLUSIONS: WHERE DO WE GO FROM HERE?

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Brock Fenton began the meeting by stating the aim of this symposium: to learn about and appreciate the use and application of bat detectors. I'm sure that everyone will agree that this aim has been achieved admirably. We have covered a range of topics based around three major themes:

- Bat-detector limitations and capabilities
- Species identification from ultrasound recordings
- Resources, research, and study

The meeting resulted in an exchange of ideas across at least three interfaces.

1) Experienced users and new users of bat detectors

We have covered a lot of applications where bat detectors can be used and have seen how much can be achieved even with simple equipment. For example, the National Bat Monitoring Programme in the UK uses inexpensive heterodyne detectors, yet the conservation value of this approach is immense. Many speakers at this symposium covered complex technical aspects of bat-detector studies with minimal jargon. I hope this has encouraged new users to grasp some of the more complex technology that is available. I encourage new users to communicate with established users when planning their studies.

Several general messages that emerged from this symposium are especially important for new users of bat detectors.

- There is no "best" bat detector or analysis package. Each method has its own costs and benefits, and these need to be carefully considered before embarking on a project. Appreciate the limitations imposed by each of the different types of detectors, but don't be put off by them.
- Complementary approaches are important. Always back up acoustic surveys with trapping studies to verify acoustic identifications and to catch species that are poorly detected because of their faint echolocation calls.
- Plan surveys carefully. Ask specific questions, formulate hypotheses, think about statistical analysis, and address issues such as replication and randomization.

2) The interface between established users

Most experienced bat researchers at this symposium came away with new ideas and will, hopefully, put into practice some of the novel approaches that have been discussed.

3) The interface between developers and users of bat detectors and sound-analysis equipment

The developers of new equipment are becoming more responsive to the needs of bat researchers, and this should further improve the wonderful technology we have at our disposal.

One of the key features of the meeting has been the interchange of ideas between American and European researchers. Historically, many researchers read only journals published on their own continent, and most American studies use Anabat detectors, while Europeans use time-expansion and heterodyne detectors. I believe the Americans here have learned much about time expansion and heterodyning, and the Europeans now realize the power of simultaneous sampling with multiple recording stations, which is possible with relatively low-cost devices like Anabat detectors. In the future, it would be good to see this interchange developing with more cross-continent communication at the North American and European Bat Research Symposia.

Among the successes of this symposium has been a strengthening of approaches in the establishment and credibility of protocols and in identifying new strategies for bat-detector surveys. We have seen the importance of considering the full repertoire of calls in developing call libraries and have learned new ways to improve the quality of our recordings (for example, by avoiding overloading and aliasing in time-expansion recordings). We have considered approaches to develop call libraries and make them accessible to a wide range of users. We were often reminded about the importance of watching our study animals. Although technology allows us to make great advances in studying the activity of bats, many speakers highlighted the importance of not getting too carried away by technology: There is no substitute for watching bats in nature.

In summary, we leave the meeting with a new appreciation for the value of bat detectors in conservation biology. Detector studies open up huge opportunities for studying biodiversity, habitat use, and the distribution of bats, especially when used in combination with other methods. Given the importance of bat detectors for studying the conservation of bats, what are the future challenges that await us? This symposium identified many issues that still need to be addressed:

- *The identification of difficult species.* Unconventional methods of picturing sound, such as those described by Joe Szewczak, can make similar spectrograms look radically different if we consider, for instance, the ways that power is distributed over time in the call.
- *The future of large-scale monitoring of species through bat-detector studies.* These will be valuable in detecting popula-

tion trends, while they also raise the challenge of coordinating these programs across large geographic areas.

- *The challenges of acoustic identification in the tropics are most severe*, because of the high species richness there. The biodiversity of these regions gives them great conservation value. The challenges are especially important given the high rates of habitat loss in tropical regions.
- *We need always to be aware of new advances in technology*, both in the processing of acoustic information and in complementary fields of bat research.
- *We need better methods for handling large quantities of acoustic data*.
- *We can improve sampling protocols* by learning more about costs and benefits of the transect methods used in bird surveys.
- *We are likely to encounter more cryptic species of bats*. Some will have different echolocation calls from their close relatives, while others will not.

This symposium developed from a Bat Conservation International workshop in Portal, Arizona, where Lars Pettersson and Herman Limpens joined BCI staff as tutors. Lars and Herman have been involved in much of the planning for this meeting, while substantial input came from Brock Fenton, Bill Rainey, and Robert Barclay, all of whom also helped moderate the discussion sessions. Thanks are due to the speakers for their excellent presentations and to those who contributed posters. The National Fish and Wildlife Foundation provided funding for the symposium.

If I were to organize a symposium on bat detector methods, I could think of no better place to hold it than Austin, Texas. The facilities have been excellent and the opportunities to see the free-tailed bats at Congress Avenue Bridge and Bracken Cave were outstanding. It is remarkable that the co-discoverer of echolocation presented new and challenging data at this meeting – his last. Don Griffin must have been amazed at the ways his discovery has led to new developments in a wide range of disciplines, from neurobiology to conservation biology. Don set an example to us all in maintaining a long-term interest in nature and in always challenging our preconceptions about bat echolocation.